



IMPERIAL AGRICULTURAL  
RESEARCH INSTITUTE, NEW DELHI.







# THE JOURNAL OF NUTRITION

VOLUME II

SEPTEMBER 1929—MAY 1930

## EDITORIAL BOARD

EUGENE F. DuBois, <i>New York City</i>	LAFAYETTE B. MENDEL, <i>New Haven, Conn.</i>
HERBERT M. EVANS, <i>Berkeley, Calif.</i>	HAROLD H. MITCHELL, <i>Urbana, Ill.</i>
ERNEST B. FORBES, <i>State College, Pa.</i>	MARY S. ROSE, <i>New York City</i>
GRAHAM LUSK, <i>New York City</i>	HENRY C. SHERMAN, <i>New York City</i>
ELMER V. McCOLLUM, <i>Baltimore, Md.</i>	HARRY STEENBOCK, <i>Madison, Wis.</i>

JOHN R. MURLIN, *Managing Editor, Rochester, New York*



*Published for*

THE AMERICAN INSTITUTE OF NUTRITION, INC.

*By*

CHARLES C THOMAS, PUBLISHER

SPRINGFIELD, ILL.



## ERRATA

In the article by Maynard and McCay, Volume II, No. 1, legends for Charts 4, page 76, and 6, page 79 should be interchanged. The sub-joined legends may be pasted below the figures to correct this error.

CHART 6. Iodine number of milk fat.

CHART 4. Average daily yield of fat.

---

## ERRATA

In Table I, page 485, Vol. II, No. 5, Ration 998 should read "1.3" under the column headed "NaCl".



# CONTENTS OF VOLUME II

## JOURNAL OF NUTRITION

No. 1, SEPTEMBER, 1929

	PAGE
EVANS, H. M., and SAMUEL LEFKOVSKY, Technique for determination of the antineuritic vitamin B. ....	1
QUINN, E. J., C. G. KING, and B. H. DIMIT, A study of the effect of certain diets upon the growth and form of albino rats. ....	7
DANIELS, AMY L., DOROTHY JORDAN, and MARY K. HUTTON, The development of the suckling young of milk fed rats. ....	19
MCQUARRIE, IRVINE, Protein metabolism of children on diets extremely low in carbohydrate. ....	31
BING, FRANKLIN C., and LAFAYETTE B. MENDEL, The vitamin B and the vitamin G requirements of the albino rat. ....	49
HELLER, V. G., and CHARLES CASKEY, An application of some of the more recent methods of estimating vitamin D. ....	59
MAYNARD, L. A., and C. M. MCCAY, The influence of a low-fat diet upon fat metabolism during lactation. ....	67
MURLIN, JOHN R., WILLARD R. LINE, H. A. PIPER, and H. B. PIERCE, The nutritive value of cereal breakfast foods. I. Composition and heat value. ....	83
CARMAN, J. S., H. G. SMITH, GLADYS C. HAVENS, and J. R. MURLIN, The nutritive value of cereal breakfast foods. II. Digestibility <i>in vitro</i> , with a study of methods. ....	91

No. 2, NOVEMBER, 1929

JONES, LOYD A., Isolation of narrow spectral regions by selective absorption. ....	111
CLAUSEN, ETHEL M. LUCE, The use of isolated radiations in experiments with the rat. I. The effect of infra-red radiation on the growth of the rachitic rat. ....	125
ROSE, WILLIAM C., and FLORENCE L. CATHERWOOD, Do baking powder residues exert injurious effects upon growth and nutrition? ....	155
SCHWARTZE, E. W., F. J. MURPHY, and R. M. HANN, Rolled oats and bran in a scurvy-producing diet and the negative control test. ....	171
HUGHES, J. S., H. F. LIENHARDT, and C. E. AUBEL, Nerve degeneration resulting from avitaminosis A. ....	183
WHITACRE, JESSIE, ALICE WILLARD, and KATHERINE BLUNT, Influence of fiber on nitrogen balance and on fat in the feces of human subjects. ....	187
McLAUGHLIN, LAURA, The nutritive value of New Zealand spinach. .	197

	PAGE
HITCHCOCK, F. A., and FRANCES R. WARDWELL, Cyclic variations in the basal metabolic rate of women.....	203

#### No. 3, JANUARY, 1930

MATTILL, H. A., and H. G. SMITH, The nutritive value of cereal breakfast foods. III. The rate of digestion and absorption as determined by experiments on rats.....	217
MITCHELL, H. H., and JESSIE R. BEADLES, The paired-feeding method in nutrition experiments and its application to the problem of cystine deficiencies in food proteins.....	225
LONG, M. LOUISA and FRITZ BISCHOFF, The effect of insulin upon the body weight of the rabbit.....	245
HONEYWELL, HANNAH E., R. ADAMS DUTCHER, and CHESTER D. DAHLE, Vitamin studies XVII. Ossifying potency of raw and evaporated milks.....	251
MORITZ, ALAN R., and CARL KRENZ, The relation of the fat-soluble vitamins A and D to the development of experimental rickets in rabbits.....	257
RUSSELL, WALTER C., The vitamin A content of yellow and white-capped yellow dent corn.....	265
COX, GERALD J., and LEONA HUDSON, The nephropathogenic action of cystine. II. The dietary control of cystine nephrosis.....	271
HART, E. B., C. A. ELVEHJEM, and H. STEENBOCK, A study of the anemia of young pigs and its prevention.....	277
FALCON-LESSES, MARK, The cause of the laxative action of bran.....	295
MURLIN, JOHN R., The emptying mechanism of the stomach.....	311

#### No. 4, MARCH, 1930

SCHWARTZ, E. W., F. J. MURPHY, and R. M. HANN, Studies on the destruction of vitamin C in the boiling of milk.....	325
AXTMAYER, J. H., A study of the vitamin B complex of red kidney beans and polished rice.....	353
CARPENTER, THORNE M., and EDWARD L. FOX, The gaseous exchange of the human subject. I. As affected by the ingestion of water at 37.0° C.....	359
CARPENTER, THORNE M., and EDWARD L. FOX, The gaseous exchange of the human subject. II. As affected by small quantities of dextrose	375
CARPENTER, THORNE M., and EDWARD L. FOX, The gaseous exchange of the human subject. III. As affected by small quantities of levulose.....	389
SANDELS, M. R., Experimental nutritional polyneuritis in the rat.....	409
SHERMAN, H. C., and H. L. CAMPBELL, Further experiments on the influence of food upon longevity.....	415
THOMAS, KARL, Biological values and the behavior of food and tissue proteins.....	419

	PAGE
MITCHELL, H. H., The significance of surface area determinations. . . .	437
MITCHELL, H. H., The surface area of single comb white leghorn chickens. . . . .	443
SUPPLEE, G. C., O. D. DOW, G. E. FLANIGAN, and O. J. KAHLENBERG, A comparative study of liquid and dry milk as anemia-producing diets. . . . .	451
HUFFMAN, C. F., C. S. ROBINSON, O. B. WINTER, and R. E. LARSON, The effect of low calcium, high magnesium diets on growth and metabolism of calves. . . . .	471
SURE, BARNETT, Dietary requirements for fertility and lactation. XXII. Further studies of the role of milk fat in fertility and lactation	485
CLAYTON, MARY M., The comparative value of different food proteins for reproduction and lactation in the rat. I Beef muscle, liver and kidney. . . . .	491
BROWN, J. B., and S. G. MORRIS, The effect of varying amounts of menhaden oil in the diet on the composition of the body fat of the white rat. The storage of highly unsaturated fatty acids. . . . .	509
MACLEOD, FLORENCE L., The chemistry of vitamin D. . . . .	517





SEPTEMBER, 1929

TECHNIQUE FOR DETERMINATION OF THE  
ANTINEURITIC VITAMIN B<sup>1</sup>

BY HERBERT M. EVANS AND SAMUEL LEPKOVSKY

*(From the Department of Anatomy—University of California, Berkeley)*

Received for Publication—April 22, 1929

IN THE studies of vitamin B reported in the literature, we find that various workers report different quantities of yeast necessary for what they term normal growth. These quantities range from .05–.7 gm daily. Some of these differences can be related to the potencies of the yeasts used. However, we feel that many of these differences are due to the composition of the basal rations used. An examination of the literature at once reveals that there is no uniformity in the character of the basal rations used for vitamin B measurements. Some workers use starch exclusively as their source of energy; some use starch and fat, while others employ sucrose in their dietary mixtures.

We have had occasion in this laboratory to make a great many measurements of materials for their antineuritic vitamin B content. Autoclaved yeast was present in all our diets, so that we were working as far as we know with only one of the vitamin B factors. We have found that the growth obtained in the absence of the antineuritic vitamin B depends largely upon the basal diet used. The following factors have been found to be of importance: (1) The amount of protein, (2) the kind and amounts of carbohydrates used, (3) amount of fat. The last named is of particular importance.<sup>2</sup>

Figure 1 shows the growth on diets, *devoid of any added antineuritic B* but varying in the form of energy or amount of protein that they carry. The poorest growth is obtained when the diet consists of sucrose and 25 per cent protein<sup>3</sup> (Diet C). When 10 per cent of fat is added to this

<sup>1</sup> Aided by grants from the Committee for Research in Problems of Sex of the National Research Council, the United States Bureau of Dairying, and the School of Agriculture and Board of Research of this institution.

<sup>2</sup> Evans, Herbert M. and Lepkovsky, S. Sparing action of fat on the antineuritic vitamin.—*Science*, September 28, 1928, LXVIII, 298.

<sup>3</sup> All references to protein include the extracted casein (L-3) plus the protein furnished by the 10 per cent autoclaved yeast. Approximately 50 per cent of the yeast is protein.

diet replacing an equal amount of sucrose (Diet E) little or no improvement results. If the sucrose is replaced with dextrinized corn starch (Diet D) slight growth improvement results. When 10 per cent of the starch is replaced with an equal amount of fat (Diet F), further improvement in

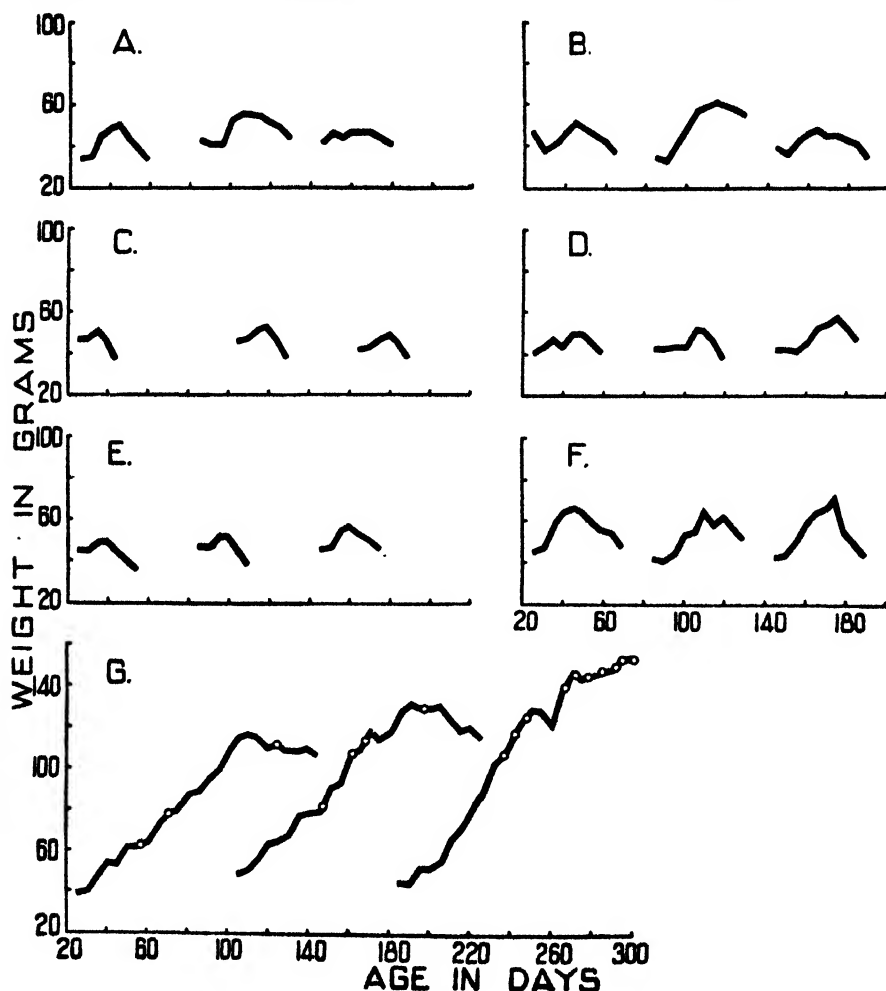


FIG. 1.—Depicts the growth of animals on diets without any added antineuritic vitamin B.

growth results. If the protein content is raised to 50 per cent (Diet A), better growth is obtained than on the diet with 25 per cent protein (Diet C), and when the amount of the protein is raised still further (75 per cent of the diet—Diet B) a slightly greater improvement in growth results. If a high per cent of fat is used with the protein (Diet G), moderately good growth is obtained without any added antineuritic vitamin B.

The animals on this diet remained in fairly good condition for six months, but one is now showing typical symptoms of beri-beri.<sup>4</sup>

Figure 2 shows the same diets *with 50 mg. brewers' yeast added as a low source of the antineuritic vitamin B*. The poorest growth here is

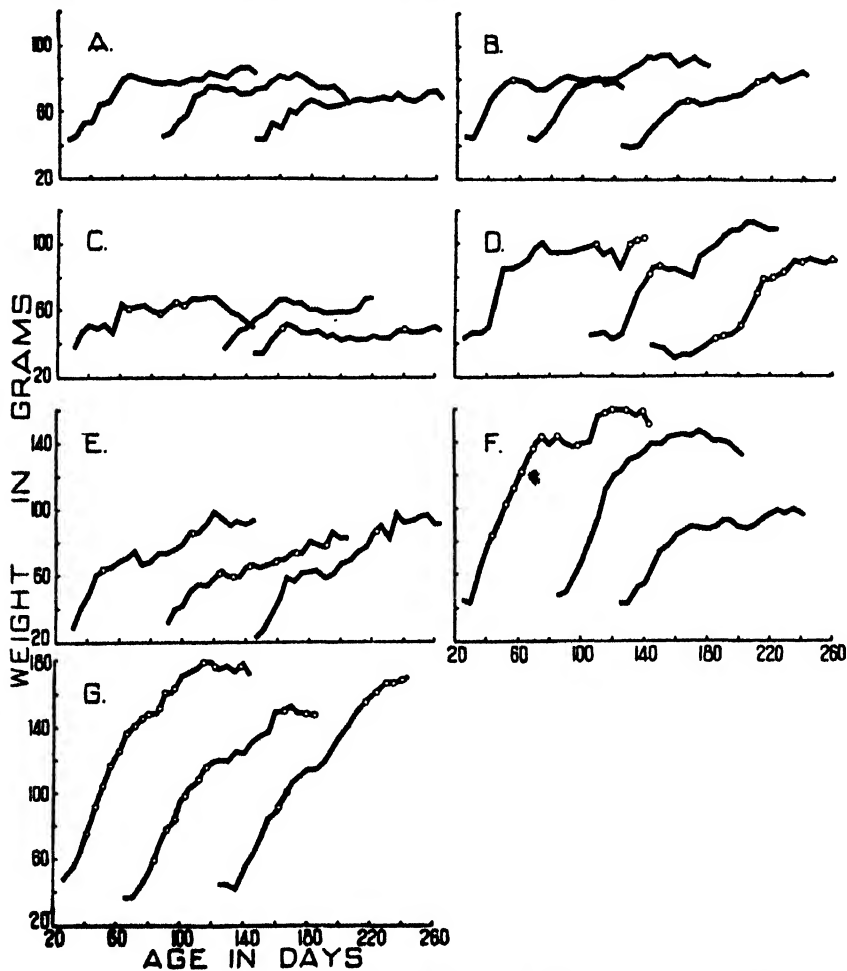


FIG. 2.—Depicts the growth of animals on the same diets used in Fig. 1 but with 50 milligrams of brewers' yeast added as a low source of antineuritic vitamin B.

again obtained on the sucrose-protein diet (25 per cent protein—Diet C). On this diet, the animals plateau at about 80 grams in weight and then slowly decline until death intervenes, usually with symptoms of beri-beri.

<sup>4</sup> The symptoms of beri-beri as seen on diets low in the antineuritic vitamin B may be described as follows: The hind limbs especially are spastic and are lifted in a stiff, high way. There are extensor thrusts of the fore and hind limbs. Movements in a circle are often observed. Ultimately paralysis of the legs supervenes.

If 10 per cent fat replaces an equal amount of sucrose (Diet E), growth is considerably accelerated. In the presence of 50 per cent fat (Diet G) very close to normal performance is observed. When corn starch replaces sucrose (Diet D) as a source of energy there is improvement in growth. When 10 per cent starch is replaced by 10 per cent fat (Diet F) there is a still greater response. If the protein is increased in a protein-sucrose diet, (Diets A and B) improvement in growth results. From these data it is easily seen how the same amount of yeast may yield different antineuritic values. It is at once evident that the diet most sensitive to antineuritic vitamin B deficiency is the sucrose-protein diet (25 per cent protein—Diet C). It would seem, therefore, to be the most suitable diet for the study of this vitamin.

Though we were not directly interested in the "balance of proteins to vitamin B" so extensively studied by Hartwell, Drummond and others,<sup>5</sup> we find that our data bear on this question (Fig. 2). When the amounts of protein and sucrose in our sucrose-protein diet (25 per cent protein—Diet C) were interchanged as was the case in Diet B, marked improvement resulted, thus indicating that added protein acted as a sparer of vitamin B.<sup>6</sup> On the other hand, when starch replaced sucrose in Diet C, as is the case in Diet D, the improvement was greater than in the high protein diet (Diet B).

#### SUMMARY

Data have been presented which show the importance of having a uniform basal diet when comparative studies of the antineuritic vitamin B are made.

The diet most sensitive for the determination of the antineuritic vitamin B has been found to be a sucrose-protein diet (25 per cent protein—Diet C).

The curves illustrate the growth of individual female rats. Litter mate sisters were distributed throughout the various groups. All the animals were placed on the diets at 21 days of age and

<sup>5</sup> Most of these studies were done with the vitamin B complex; Hartwell, G. A., *Biochem. Jour.* xviii, 785 (1924); Reader, V. and Drummond, J. C., *Biochem. Jour.* xx, 1256 (1926); Sherman (*Jour. Biol. Chem.* lxxiv, 117 (1927)) writes "no basis is found for any belief that the vitamin B requirement is influenced by the protein intake in the age period covered by these experiments."

<sup>6</sup> This interpretation of the data must be taken with due caution. Though our casein was washed thoroughly to remove antineuritic vitamin B, we cannot be certain that this has been entirely accomplished (Palmer, L. S. and Kennedy, C., *Jour. Biol. Chem.* lxxiv, 591 (1927)). and hence improvement may be due to unextracted B residual in the casein. Our experience with our basal sucrose-protein diet (25 per cent protein—Diet C), either does not admit the possibility of residual antineuritic B in our casein, or, if such minute impurities do exist, they are evidently without noticeable influence in our studies. Furthermore, it should be emphasized that we worked only with one level of antineuritic vitamin B (50 mg. yeast) and we have no reason to believe that our observations at this level will necessarily hold for other levels of the vitamin.

maintained in cages with wire mesh floors. The animals were watched daily for breakdown of the vaginal closing membrane and thereafter for oestrous changes in the vaginal smear indicative of ovulation. The small circles interrupting the growth curves in the charts indicate times of ovulation.

COMPONENTS OF THE DIETS USED IN THE EXPERIMENTS REPRESENTED  
IN FIGURES 1 AND 2.

<b>A—Sucrose-Protein 50%</b>		<b>B—Sucrose-Protein 75%</b>	
Casein (L-3) . . . . .	45	Casein (L-3) . . . . .	70
Sucrose . . . . .	45	Sucrose . . . . .	20
Salts . . . . .	4	Salts . . . . .	4
Autoclaved yeast . . . . .	10	Autoclaved yeast . . . . .	10
<b>C—Sucrose-Protein 25%</b>		<b>D—Starch-Protein 25%</b>	
Casein (L-3) . . . . .	20	Casein (L-3) . . . . .	19
Sucrose . . . . .	70	Dextrinized cornstarch . . . . .	67
Salts . . . . .	4	Salts . . . . .	4
Autoclaved yeast . . . . .	10	Autoclaved yeast . . . . .	10
<b>E—Sucrose-Lard-Protein 24%</b>		<b>F—Starch-Lard-Protein 24%</b>	
Casein (L-3) . . . . .	20	Casein (L-3) . . . . .	20
Sucrose . . . . .	59	Dextrinized cornstarch . . . . .	59
Salts . . . . .	4	Salts . . . . .	4
Autoclaved yeast . . . . .	10	Autoclaved yeast . . . . .	10
Lard . . . . .	10	Lard . . . . .	10
<b>G—Lard-Protein 41%</b>			
Casein (L-3) . . . . .	36		
Salts . . . . .	4		
Autoclaved yeast . . . . .	10		
Lard . . . . .	50		

All diets were supplemented with two drops of cod liver oil daily.

Casein (L-3)—Commercial casein (Golden State Milk Products Company) which has been precipitated from milk with hydrochloric acid, dried and delivered to us in sacks, was extracted in wooden tubs with acidulated water (40 cc. glacial acetic acid to 40 liters of filtered tap water). The acidulated water was siphoned off and replaced twice daily for six days. On the seventh day the casein was washed twice with distilled water replacing the acidulated tap water. The water was thoroughly drained from the casein and the casein washed once with 50-80 per cent alcohol and once with 85-95 per cent alcohol followed by one ether treatment. It was then spread in pans and the ether allowed to dissipate at room temperature under a fan.

Autoclaved yeast—Whole dried yeast (bakers') was generously supplied us by the Fleischmann Company of New York. It was spread in pans to a depth of less than one inch and autoclaved for five hours at 18 to 20 pounds pressure. This yeast was added as the source of the thermostable water-soluble factor throughout the experiment. Abundant trials showed it to be almost entirely, if not absolutely, free from antineuritic B.

Brewers' yeast—A whole, dried brewers' yeast of high potency was used as the source of the thermolabile or antineuritic factor B. It was generously furnished by the Vitamin Food Company through Dr. Edward A. Rumley of New York.

Dextrinized corn starch was prepared by pouring cold starch paste into boiling water, boiling for 5 minutes, spreading in thin layers in aluminum pans and drying at 110-120° C.

Salt mixture #185 after McCollum—Sodium chloride 51.0, crystals of magnesium sulphate 159.6 monobasic sodium phosphate 104.1, monobasic calcium phosphate 162.0, dibasic potassium phosphate 286.2, ferric citrate 35.4 and calcium lactate 390.0.



---

---

# A STUDY OF THE EFFECTS OF CERTAIN DIETS UPON THE GROWTH AND FORM OF ALBINO RATS

BY E. J. QUINN, C. G. KING AND B. H. DIMIT

(*From the Department of Chemistry, Columbia University,  
New York City*)\*

Received for Publication—April 24, 1929

MUCH study has been given in the past to the effects of deficient diets on the skeletal growth of the animal body. Jackson (1924) has reviewed the work that has been accomplished along this line up to 1924, while more recently Winters, Smith and Mendel (1927) have published the results of a study of the effects on growth of animals of a low calorie diet, of inadequate protein, and of inadequate salt intake. No reference, however, has been found regarding the effect of a vitamin deficiency on general skeletal growth.

One of the purposes of the present investigation was to study the effects of a deficiency of vitamin A or vitamin B upon the development of the body of albino rats, the specific purpose being to determine in what way the girth of the chest, the width of the hips and the size of the long bones of the legs of such animals compared with corresponding measurements of normal animals of the same age. The term vitamin B as used in this paper covers both the antineuritic substance and the more heat-stable substance now known as the antipellagric vitamin (vitamin B<sub>2</sub> or G).

The normal rats used for comparative purposes in this study were from diet No. 13 (Diet B), consisting of one-third whole milk powder, two-thirds whole wheat and sodium chloride two per cent of the wheat.

At the time the investigation was started there were available rats from another adequate diet, No. 16 (Diet A) consisting of one-sixth whole milk powder, five-sixths whole wheat and sodium chloride two per cent of the wheat. Although the animals receiving Diet A were apparently normal, they differed somewhat from those receiving Diet B. Earlier experiments (Sherman and Campbell 1924) have shown the latter animals to be considerably heavier at any given age, to have a longer life-span, to have a better reproduction record, and to be less subject to lung infection. A study of the calcium and phosphorus content (Sherman and MacLeod 1925), (Sherman and Quinn 1926) of rats from these two diets has also shown retardation of calcification in the animals on diet 16 (Diet A) during

---

\* Published as contribution No. 605 from the Department of Chemistry, Columbia University.



**TABLE I**  
**CERTAIN BODY AND BONE MEASUREMENTS OF ALBINO RATS ON ADEQUATE AND VITAMIN DEFICIENT DIETS**  
**MALES**

Diet No.	Average Age days	Maximum Weight Average grams	Final Weight Average grams	Trunk Length Average cm.	Chest Girth Average cm.	Femur			Tibia		Hip Width Average cm.	Humerus		Radius Length Average cm.	No. Animals
						Length Avg. cm.	Dia. Avg. cm.	Length Avg. cm.	Dia. Avg. cm.	Length Avg. cm.		Dia. Avg. cm.			
13	29	47	47	12.2	7.4	1.86	.20	2.16	.15	.98	1.57	.16	1.47	10	
	61	135	135	17.2	8.7	2.66	.25	3.03	.19	1.44	2.11	.20	1.96	13	
	95	226	226	20.3	11.3	3.15	.28	3.45	.21	1.68	2.48	.71	2.27	7	
	132	272	272	21.7	11.7	3.44	.30	3.82	.21	1.81	2.70	.22	2.42	8	
	225	295	295	22.6	12.8	3.61	.32	3.93	.22	1.96	2.86	.23	2.53	11	
	426	292	284	22.6	12.8	3.61	.32	4.00	.24	1.94	2.88	.23	2.61	21	
16	30	42	42	11.7	5.9	1.80	.19	2.16	.14	.88	1.52	.16	1.45	6	
	60	105	105	15.4	7.6	2.40	.22	2.75	.18	1.22	1.95	.19	1.78	7	
	95	197	197	19.2	10.7	3.01	.28	3.27	.20	1.58	2.30	.20	2.14	12	
	130	235	235	20.7	13.3	3.29	.29	3.61	.23	1.73	2.55	.21	2.33	10	
	224	273	273	21.7	12.9	3.51	.31	3.78	.22	1.87	2.74	.21	2.44	13	
	473	275	226	21.6	11.9	3.45	.32	3.71	.24	1.86	2.69	.23	2.45	18	
B 107 B'	84	76	64	15.4	6.4	2.50	.23	2.90	.18	1.21	1.99	.16	1.80	18	
	84	97	96	16.7	7.5	2.65	.25	3.06	.18	1.31	2.10	.16	1.90	26	
A 379a A'	73	104	75	15.9	6.6	2.63	.25	3.03	.19	1.29	2.05	.20	1.90	5	
	104	109	108	16.6	7.9	2.67	.24	3.10	.19	1.29	2.08	.20	1.94	24	

TABLE I (continued)  
FEMALES

13	30	43	43	12.0	6.3	1.86	.19	2.17	.15	.92	1.58	.17	1.45	7
	60	111	111	16.4	8.2	2.58	.23	2.91	.19	1.39	2.04	.19	1.88	9
	99	199	199	19.4	10.5	2.99	.29	3.30	.20	1.61	2.40	.20	2.18	2
	212	215	215	20.2	12.4	3.25	.30	3.57	.21	1.80	2.53	.21	2.36	8
	444	217	214	20.5	11.8	3.30	.30	3.65	.22	1.75	2.62	.21	2.45	35
16	30	41	41	11.4	5.5	1.75	.19	2.11	.13	.88	1.50	.16	1.43	5
	60	89	89	15.1	7.4	2.29	.20	2.54	.16	1.20	1.81	.18	1.61	3
	94	152	152	17.7	9.6	2.86	.23	3.11	.20	1.48	2.22	.19	2.07	4
	184	193	193	19.1	13.0	3.17	.29	3.40	.24	1.68	2.43	.22	2.23	2
	478	207	194	19.6	11.8	3.19	.30	3.40	.23	1.67	2.45	.22	2.31	22
B 107 B'	84	71	57	14.9	6.1	2.42	.23	2.81	.18	1.17	1.95	.15	1.78	16
	84	91	90	16.2	7.5	2.57	.23	2.95	.18	1.28	2.05	.16	1.88	18
A 379a A'	84	88	66	15.1	5.9	2.49	.22	2.92	.17	1.17	1.91	.19	1.86	3
	105	98	97	15.9	7.7	2.57	.24	2.95	.19	1.22	2.01	.19	1.88	22

B—Animals in this group made average gains in weight of 6-11 grams during an 8 weeks' experimental period. B'—Animals in this group made average gains in weight of 50-55 grams during an 8 weeks' experimental period.

A—Animals in this group received from weaning or 28 days of age until their death the vitamin A deficient diet only. A'—Animals in this group made average gains in weight of 22-30 grams during an 8 weeks' experimental period.



TABLE II (continued)  
FEMALES

(Diet No. 13)

30	43	3.6	6.8	23.1	19.8	46.7	27.2	29.7	1.90	6.45	5.52	13.0	7.59	8.22	9.78	14.5	9.29	7
60	111	6.7	13.5	43.2	38.1	79.9	54.4	59.0	2.00	6.36	5.63	11.8	8.04	8.72	11.2	15.3	10.7	9
99	199	10.3	19.0	66.6	60.3	124	82.9	91.3	1.85	6.49	5.87	12.0	8.08	8.89	10.3	16.5	12.0	2
212	215	10.7	17.3	66.2	60.1	119	84.9	91.1	1.61	6.21	5.65	11.3	7.98	8.56	10.8	17.0	12.1	8
444	217	10.6	18.4	65.8	59.5	124	82.8	88.6	1.74	6.21	5.62	11.7	7.82	8.37	11.0	16.6	12.5	35

(Diet No. 16)

30	41	3.6	7.5	23.4	19.4	46.6	27.3	28.7	2.07	6.51	5.40	13.0	7.60	7.97	9.21	16.2	9.38	5
60	89	5.9	12.0	38.9	35.0	74.1	49.2	55.3	2.04	6.59	5.94	12.6	8.34	9.38	11.5	15.9	10.1	3
94	152	8.6	15.8	53.1	48.9	103	68.5	73.4	1.84	6.19	5.69	12.0	7.97	8.55	12.4	15.5	11.7	4
184	193	10.1	14.8	60.9	56.8	115	79.4	86.5	1.47	6.03	5.62	11.4	7.86	8.57	10.9	14.2	11.0	2
478	207 <sup>a</sup>	10.6	17.5	64.9	60.8	124	84.4	89.6	1.56	6.14	5.76	11.7	8.00	8.48	10.6	14.9	11.1	22

(Diet No. 107)

84	71 <sup>a</sup>	4.8	11.6	29.3	25.3	60.7	36.4	39.9	2.44	6.16	5.30	12.7	7.64	8.37	10.5	15.6	13.0	16
84	91	5.7	12.1	35.4	30.8	71.1	44.3	48.5	2.16	6.30	5.49	12.7	7.90	8.61	11.1	16.4	12.8	18

(Diet No. 379a)

84	88 <sup>r</sup>	5.8	14.9	35.3	30.1	75.2	46.1	47.3	2.56	6.06	5.17	12.9	7.91	8.12	11.3	17.1	10.1	3
105	98	6.2	12.7	38.1	33.2	80.3	48.8	52.1	2.06	6.19	5.39	13.0	7.91	8.46	10.7	15.5	10.6	22

<sup>1</sup> Avg. Final Wt. 284 gms. Final Wt. = 12.6; Chest Girth = 22.2  
<sup>2</sup> " " 226 " " = 10.5;  
<sup>3</sup> " " 64 " " = 4.2;  
<sup>4</sup> " " 75 " " = 4.7;

<sup>5</sup> Avg. Final Wt. 194 gms. Final Wt. = 9.9; Chest Girth = 16.4  
<sup>6</sup> " " 57 " " = 3.8;  
<sup>7</sup> " " 66 " " = 4.4;

the period of greatest growth. This was found (Sherman and MacLeod 1925) to be due to a lower mineral intake and not, primarily, to a lower intake of the fat-soluble vitamins.

In view of the above differences it was thought of interest to include, in this study of the effect of diet on skeletal growth, a comparison of animals on diet 16 (Diet A) with animals of the same age on diet 13 (Diet B) and to determine whether such moderate but well defined differences as had been found in respect to the general well being of the animals on the two diets would be accompanied by differences in body form and development.

The technique employed in making the various measurements that are discussed in this paper is in brief as follows: Length of body and chest measurements were taken immediately after the animal was chloroformed. All other measurements were made as soon as possible after the death of the animal. With the animal pinned back downward, with nose and tail extended but not under tension, the trunk or body length was determined by measuring with calipers the distance from the tip of the nose to the anus. Chest girth measurements were taken by wrapping a cotton string around the chest at the place of smallest girth and pulling taut but avoiding wrinkling of skin. This string length was then carefully measured with a metric rule. Hip width was taken as the distance between the hip sockets and was determined with calipers resting on the ventral sides of the two cups. The leg bone dimensions were determined with calipers adjusted to fixed points on the sides or ends of each bone. Both right and left leg bones of each animal were measured but no differences in average values were found between them, the measurement of one side simply serving as a check on the other.

The average values resulting from the measurements described above are recorded in Table I. In Table II are given the calculated ratios of the weight of animal to their length of body, chest girth, hip width and length of long bones of legs; also their length of body to girth of chest, hip width, and length of long bones of legs; and length of certain leg bones to their diameter. The weight ratios of Table II are based in each case, with the exceptions noted, on the maximum weight of the animals. The data whose average values appear in Table II were treated statistically when the group contained sufficient numbers to warrant this. The values thus obtained have served in confirming the judgement of the experimenters in the conclusions that have been drawn in this paper. Because of lack of space, the results of the statistical treatment are not herein reported.

The small number of animals examined in many of the younger age groups studied is considered to justify only the conclusion that these points serve as an index of the trend of these particular groups. Variations in the smaller groups however, are somewhat compensated for by their relationship to other groups on the same diet. The number of animals retarded in growth over a period of 8 weeks by a deficiency of vitamin A or vitamin B, and of those over one year of age on diets 13 and 16, is thought to be sufficient to establish these points fairly satisfactorily. It will be noticed that the changes in body form recorded for the different groups are more marked when animals of similar age are compared than when comparison of animals of similar weight is made. Both of these variables should therefore be kept in mind when interpreting the data given in the accompanying tables. Providing sufficient vitamin is added, the vitamin-deficient diets used in this study are adequate for apparently normal growth, although the mineral content is obviously different from that of either diet 13 or 16.

To facilitate discussion of the various groups and to enable comparisons to be more clearly presented, the results of the investigations are considered under the several headings given below.

#### ANIMALS RESTRICTED IN GROWTH BY A DIET DEFICIENT IN VITAMIN A

Rats from vitamin A experiments recently conducted in this laboratory have furnished excellent material for the study of skeletal growth relationships of animals stunted in growth by a deficiency of vitamin A. Eight animals (negative controls) had been kept on a basal vitamin A-free diet from 28 days of age until their death; 46 animals had been continued on the A-free diet from weaning until the surplus stored vitamin of their bodies was depleted, at which time the basal diet was supplemented by such carefully weighed daily portions of whole milk powder that an average gain in weight of 22-30 grams resulted during an 8 weeks period. This approximates what Sherman and Munsell (1925) have recommended as the rate of growth to be employed in quantitative studies of vitamin A.

The basal vitamin A-free diet referred to (No. 379a) consists of casein 18 per cent, corn starch 67 per cent, yeast 10 per cent, Osborne and Mendel's salt mixture 4 per cent, and sodium chloride 1 per cent.

From a study of the ratios in Table II it is found that the length of the body, girth of chest and width of hip of the vitamin A-deficient animals increased during the time of restricted feeding to a relatively greater extent than did the weight of such animals during this period.

Ratios of weight to each of these measurements are lower than the corresponding ratios for normal rats of the same age.

Growth in length of the long bones of the legs also continued in animals on the vitamin A-deficient diets, and the ratios of body weight to length of femur, tibia, scapula, humerus, and radius are all found to be lower than the ratios for diet 13 animals of the same age.

Relative to the length of the body, the chest girth of the vitamin A-restricted animals making average gains in weight of 22-30 grams over an 8 weeks period, showed no significant difference from that of normal animals of the same age or weight. In proportion to their body length, these animals have slightly longer leg bones than do normal animals of either the same age or of the same weight. The hip width is relatively narrow when comparison is made with the corresponding measurement of normal animals of the same age, but the difference is not so apparent when comparison is made with normal animals of the same weight. It has been noted that an arching or bowing of the spine is often found in rats stunted by deficient diets (Jackson 1915), (Aron 1914). It is possible that ratios involving the length of the body may be in slight error due to such a condition, but it is probably not an error that would vitiate the measurements in the case of the animals remaining in good health and making the rate of growth described above.

With the possible exception of the humerus, the relative size of the femur, tibia and humerus as shown by the length-to-diameter ratios for each of these long bones is not significantly different from the normal. Although, as has been shown above, the long leg bones of the vitamin A-restricted animal have continued to grow in length relatively faster than the body weight has increased, they have likewise thickened or increased in diameter at relatively the same rate so that quite normal symmetry of these bones results. The humerus shows a tendency to be relatively thicker than the normal in both males and females on the restricted diet, when comparisons are made between animals of the same age, but when comparisons are made between animals of approximately the same weight, the length-to-diameter ratios for the humerus of the animals on the vitamin A-deficient diets are practically the same as the ratio for the normal animal.

#### ANIMALS RESTRICTED IN GROWTH BY A DIET DEFICIENT IN VITAMIN B

Vitamin B, as the term is here used, is the mixture of the antineuritic substance and vitamin B<sub>1</sub> or G, both being necessary for growth.

For a period of 8 weeks following weaning (28 days of age), thirty-four animals were fed a basal diet free of vitamin B, plus daily weighed portions

of whole milk powder to supply sufficient vitamin B to allow the animals just to maintain their weight during an experimental period of 8 weeks. Forty-four animals were fed from weaning age the basal vitamin B-free diet supplemented with such amounts of whole milk powder that an average gain of 50–55 grams resulted in 8 weeks. The vitamin B-free diet No. 107 consists of casein 18 per cent, butter-fat 8 per cent, cod liver oil 2 per cent, Osborne and Mendel salt mixture 4 per cent, and corn starch 68 per cent. This diet has been found adequate for normal weight development when sufficient vitamin B is supplied. Previous work in this laboratory has shown that milk is relatively richer in vitamin B<sub>2</sub> or G than in the antineuritic vitamin, hence the latter was the “first limiting factor” in these experiments.

Relative to their body weight, both the animals restricted in growth to barely more than net maintenance, and those making moderate growth during the 8 weeks experimental period, showed longer bodies, greater chest girth, and hip width, and longer leg bones than did normal animals of the same age.

Relative to their body length, the vitamin B-deficient animals were found to have smaller chests than did normal animals of the same age or the same weight. Like the vitamin A-deficient animals, those on the vitamin B-restricted diets showed a tendency to have longer leg bones in proportion to the trunk or body length than did the normal.

The humeri of these animals are longer relative to the diameter than is the case with the other leg bones. It will be seen from Table I that although a definite lengthening of the humerus has taken place during the time the animals were on the vitamin B-deficient diets, the diameter of this bone is practically the same as was found for the normal rats 60 days younger. If we compare the size of the humerus of the animals on the diets low in vitamin B with the size of the same bone from normal animals of the same weight, the difference is even more pronounced than when animals of the same age are compared, the humeri of the vitamin B restricted animals being considerably longer relative to the diameter than is the case with normal animals of the same weight. A condition of bone growth is therefore indicated in these vitamin B-restricted animals that is not common to the other groups studied.

#### ANIMALS ON DIET 16

The animals on diet 16 that were used in this study had been on this diet for from nine to fifteen generations (average twelve); those on diet 13 for from one to twenty-one generations (average nine), with the animals



in each age group averaging at least three generations. They were all taken from the same original stock and all were raised under identical laboratory conditions. In proportion to their weight the young animals from diet 16 have generally longer bodies than do animals from the better diet, No. 13. In the adult animals, however, there is no significant difference in this respect. There appears to be little or no difference in the girth of chest nor in the width of hip, relative to their body weight, of the animals of the same age, or of the animals of the same weight on these two diets.

The young animals on diet 16 generally show longer leg bones in relation to body weight than were found in the case of the diet 13 animals, but for the mature animals, or those over one year of age, the ratios involving these measurements are not significantly different.

In proportion to their body length animals on diet 16 show a tendency when young to have relatively smaller chests and narrower hips than do the young on diet 13 when compared on an age basis, but the difference is insignificant for animals of the same weight. In adult life there appears to be little if any difference in the shape of the body of animals from the two diets.

There is no significant difference from the normal in the length of the tibia, radius, femur, scapula or humerus in relation to the body length of the animals on diet 16 although when compared with the normal there appears to be a slight difference in the size of the tibia, humerus and femur in the very old rats on diet 16. A tendency was found for these bones in animals over one year of age to be slightly thicker or larger in diameter in relation to their length.

It is quite significant that the ratios of measurements of body and bones of animals on the two diets, differing as do diets 13 and 16, should yield results so nearly alike. The small differences found in bone and body form of the rats from the two diets stand in contrast to the larger differences that have previously been found in the life span, reproductivity and resistance to lung infection of the animals on these two diets. Apparently a difference in diet may affect an animal in a serious and subtle way and still cause no marked effect on body form and stature.

#### SUMMARY

A study has been made of the effects of certain diets on the form and development of albino rats.

Compared with normal animals of the same age, measurements show that in the case of animals receiving a deficient supply of vitamin A there

resulted a lesser retardation of increase in the length of body and of leg, the chest girth, and the width of hips than in the body weight. In proportion to their body length, those animals which received a small amount of vitamin A food, and made gains in weight of 22-30 grams in 8 weeks, show about the same chest girth as normal animals. The leg bones of animals on the vitamin A-deficient diets were found to be relatively longer in proportion to their body length than the normal. With the exception of the humerus, the long leg bones showed no marked difference from the normal in size or shape. The humerus showed a tendency to be thicker in relation to its length, than was found in the case of the bone of normal animals of the same age, but there was practically no difference when comparisons were made between animals of the same weight.

The body form of albino rats stunted by a deficiency of vitamin B is also different from the body form of normal animals of the same age. Compared with normal animals of the same age, the animals retarded in growth because of a deficiency of this vitamin show, as in the case of the vitamin A-deficient animals, that there was a lesser retardation of increase in the length of body and legs, chest girth and width of hips than in body weight. In proportion to the length of the body, these animals have smaller chests and show a tendency to have longer leg bones relative to their body length, than do normal animals of the same age. The humeri, especially, are relatively long and slender compared with the normal.

A study was also made of the development of animals from a stock which for generations had received diet 16, a diet somewhat poorer than the normal, but one that earlier work has shown to be adequate for growth and reproduction through many generations. In the case of the mature animals, and here the larger numbers used in the comparison make the values of more significance, there appears to be no consistent difference in body trunk form of the animals on diets 16 and the better diet, diet 13.

The younger animals on diet 16 generally show longer leg bones relative to body weight than was found in the case of diet 13 animals of the same age, but the differences were negligible for animals of the same weight. The tibias, femurs, and humeri of the very old animals on 16 show a tendency to be slightly thicker or greater in diameter relative to their length than is the case with the very old rats on diet 13.

The small differences that have been found in the bone and body structure of rats on diets 13 and 16 stand in contrast to the larger differences that have previously been found in the life span, reproductivity and resistance to lung infection of the animals on these two diets.

**ACKNOWLEDGEMENT:** The authors of this paper desire to express their most sincere thanks to Professor H. C. Sherman for his many helpful suggestions during the course of this work.

#### BIBLIOGRAPHY

- Aron, H., 1914, *Berlin Klin. Wochenschr.*, XI, 972.  
Jackson, C. M., 1915, *Jour. Exp. Zool.*, XIX, 99.  
Jackson, C. M., 1924, *Inanition and Malnutrition*. Blakiston, Philadelphia.  
Sherman, H. C., and Campbell, H. L., 1924, *Jour. Biol. Chem.*, LX, 5.  
Sherman, H. C., and MacLeod, F. L., 1925, *Jour. Biol. Chem.* LXIV, 429.  
Sherman, H. C., and Munsell, H. E., 1925, *Jour. Amer. Chem. Soc.*, XLVII, 1639.  
Sherman, H. C., and Quinn, E. J., 1926, *Jour. Biol. Chem.*, LXVII, 667.  
Winters, J. C., Smith, A. H., and Mendel, L. B., 1927, *Amer. Jour. Physiol.*, LXXX, 576.



## THE DEVELOPMENT OF THE SUCKLING YOUNG OF MILK FED RATS

BY AMY L. DANIELS, DOROTHY JORDAN, AND MARY K. HUTTON

(*From the Department of Nutrition, Child Welfare Research  
Station State University of Iowa, Iowa City.*)

Received for Publication—May 11, 1929

RECENTLY Sure (1), Evans and Burr (2), and Macy, Outhouse, *et al.* (3), have called attention to the fact that the Vitamin B-requirement for lactation is considerably higher than that needed for growth. In nutrition studies, in which the criterion of the efficiency of a given food has been the production and growth of young, these investigators have pointed out that failure in normal development, and ultimate death in many cases, have been due to an insufficient amount of the antineuritic vitamin in the milk secretion. This vitamin deficiency is manifested in the suckling young by posterior paralysis, muscle incoordination, screaming, running fits, and sudden collapse. On post-mortem examination, the stomachs were found to be full of curd. Hemorrhages of the bones, especially at the junction of the occipitals and parietals were frequently observed. In the mother there was a marked loss in weight during the lactation period amounting to 20 and 30 per cent of the original weight (4).

That the antineuritic vitamin content of woman's milk, under certain conditions, may be below the physiological requirements of the human infant is well known. In beri-beri the untoward effects of the vitamin deficiency in the nursling are much more marked than in the mother. Even in seemingly adequately nourished women, it appears that the Vitamin B-content of the milk may be lower than has hitherto been appreciated. Macy, Outhouse, *et al.* (5), have observed that from 25 cc. to 30 cc. of human milk were sufficient to furnish the vitamin B-requirement in young rats, whereas 35 cc. were necessary to produce continued growth in larger animals. At this level the animals reproduced, but lactation was unsuccessful due, the authors believe, to a deficiency in the antineuritic vitamin content of the milk. For successful lactation, it appears that from 3 to 5 times as much of this vitamin is needed as for growth. The authors conclude that "the average healthy mother is producing a milk exceedingly low in Vitamin B."

There are few data available relative to the antineuritic vitamin content of cow's milk. Hopkins (6) obtained normal growth in young rats which

were receiving, in addition to a purified ration, 2 cc. of milk as the sole source of vitamin B. Kennedy and Dutcher (7) found 10 cc. per day of either summer or winter milk adequate for the rat; whereas Osborne and Mendel (8), and Johnson (9) were unable to secure comparable growth with less than 16 cc. of cow's milk. Gibson and Concepcion (10), working with polyneuritis in chickens, pigs and dogs, concluded that milk is a poor source of the antineuritic vitamin. Outhouse, Macy, *et al.* (11), obtained excellent growth in young rats during a period of twelve weeks when from 20 to 25 cc. of cow's milk was the only source of the vitamin. Growth then became less satisfactory. The addition of 0.4 gms. of fresh dried yeast brought about an immediate response in growth, whereas the addition of the same amount of autoclaved yeast was without effect. These results indicate that it is the thermolabile fraction which is the limiting factor in preventing the animals from attaining the average adult size on the milk diet. Somewhat similar results were obtained by Sure with dried milk (12). A ration consisting of 50 per cent of dried milk, as the only source of the antineuritic vitamin, produced adequate growth, but failed to promote normal lactation in rats. The addition of a concentrated extract of wheat embryo, made by extracting the embryo with 25 per cent alcohol, or the addition of a dehydrated yeast or yeast concentrate resulted in an immediate response in mammary secretion, demonstrated by the improved well being of the young. These observations led the author to conclude that cow's milk may be so lacking in the antineuritic vitamin as to be inadequate at times for the human infant.

The reported low antineuritic vitamin content of cow's milk suggested that the high mortality of the suckling young of milk-fed rats may be due in part to a too low concentration of this vitamin in the mother rat's milk. If 60 per cent of the vitamin of the food is dissipated in the metabolism and transfer to the milk (13), it seems possible that there may be too little left for proper nourishment of the suckling young. In our experience, however, the young of these milk-fed rats have never manifested the untoward symptoms characteristic of the antineuritic vitamin deficiency.

In order to test this point, we have transferred stock-fed rats to milk diets just previous to, or just following, parturition. These diets have included milk "sterilized" by boiling or pasteurizing, raw milk, and boiled, irradiated milk, as well as several superheated milk preparations, in some of which the destruction of considerable amounts of the antineuritic vitamin has been demonstrated (14). In a certain number of cases

there were added to the milk, substances known to be rich in the anti-neuritic vitamin. These have included dehydrated yeast, an autolyzed yeast concentrate and an alcoholic (80 per cent) extraction of wheat embryo. In order to rule out the possible stimulating effect of other substances in these, autoclaved autolyzed yeast concentrate, and autoclaved

TABLE I  
GROWTH OF SUCKLING YOUNG OF MILK FED RATS

Food*	Date	No. born	Wt. of litter of 4 at 4 days	Wt. of litter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
			gms.	gms.	gms.	per cent	
Milk. . . . .	1/31	13	26	132	10.3		
Milk. . . . .	2/8	6	20	45	2.4		1 died 19th day
Milk. . . . .	3/25	9	28	92	6.2		1 died 13th day
Milk + dextrin-maltose I 10%..	6/1	7	30	128	9.5	+ -0	
Milk. . . . .	6/3	9	26	144	11.4	+6.2	
Irradiated milk.	6/11	7	36	132	9.3	-7.7	Irradiated 20 min. at 2 ft.
Irradiated milk.	6/13	10	32	138	10.3	-4.5	Irradiated 20 min. at 2 ft.
Milk. . . . .	6/12	6	28	106	7.6	-2.2	
Raw milk. . . . .	6/11	6	38	114	8.8	-16.3	Mother killed young 19th day
Milk. . . . .	7/1	8	28	44	2.3	-5.4	Young died on 16th day
Milk. . . . .	11/7	7	24	86	9.4	-3.4	1 died on 19th day
Milk, Past. . . . .	9/15	9	34	72	3.9	+ -0	
Milk, Past. . . . .	11/13	10	40	158	11.4	-21.4	
Milk. . . . .	1/31	5	28	128	9.7	+2.1	Second generation
Average. . . . .			29.8	107.7	8.03	-4.73	

\* All milk was quickly boiled unless otherwise specified.

wheat embryo extract also have been tested. Data pertaining to the development of the suckling young of rats receiving milk to which was added copper sulphate, the ash of wheat embryo, and soy beans respectively, as well as those receiving our stock ration, have been included for comparison. In a few cases the results of the second generation on a given food are reported. To all milk diets small amounts of iron citrate and potassium iodide were added.

In general, the number of young in each litter was reduced to four when the young were four days of age; with a few groups the litters were reduced within twenty-four hours after birth. This seems to have had an

TABLE II  
GROWTH OF SUCKLING YOUNG OF RATS FED SUPERHEATED MILKS

Food	Date	No. born	Wt. of litter of 4 at 4 days	Wt. of litter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
			gms.	gms.	gms.	per cent	
Desiccated protein milk....	3/2	9	28	148	11.4	+2	
Desiccated protein milk+0.01 gm. $\text{Ca}_3(\text{PO}_4)_2$ +2 drops of cod liver oil.....	2/2	8	41	116	13.12	—	
Dried Milk No. No. I*.....	3/28	8	48	166	11.4	—10.8	
Dried milk No. I*+.01 gm. $\text{Ca}_3(\text{PO}_4)_2$ +2 drops cod liver oil.....	4/26	9	36	114	7.6	—11.3	
Evap. milk.....	4/24	4	44	174	12.6	—3.9	
Evap. milk.....	5/6	6	30	130	9.9	+3.9	
Evap. milk.....	5/11	9	40	197	15.3	—4.	One killed at 13 days
Evap. milk.....	5/12	9	42	174	12.8	—3.	
Evap. milk+10 cc. wheat embryo extract.	2/20	4	28	90	6.03	—14.8	
Evap. milk+0.1 gm. $\text{Ca}_3(\text{PO}_4)_2$ +2 drops cod liver oil.....	4/27	8	31	196	16.	+—0	
Dried milk No. 2†+16% butter-fat..	11/13	6	40	182	13.8	+—0	
Dried milk No. 2†+16% butter-fat.....	1/31	5	28	162	13.03	+2	Second gen. Boiled milk
Av.....			36	154	11.9	—3.6	

\* Milk dried by the spray process.

† Milk dried by the roller process. 16% of butter fat was added to make the composition comparable to Dried milk I.

influence on the results. In a few instances the number raised was less than four, owing to the death of one or two of the group. In these the estimated weight is based on the performance of the two or three surviving. The animals were weighed every three days.

Our criteria of the efficiency of the food have been: 1—the average gain per rat per week, between the fourth and twenty-second day; and 2—the percentage of weight gain or loss in the mother during the lactation period. The results are given in the tables.

TABLE III  
GROWTH OF SUCKLING YOUNG OF RATS FED MILK+AUTOLYZED YEAST

Milk <i>ad lib.</i> + autolyzed yeast per day	Date	No. born	Wt. of lit- ter of 4 at 4 days	Wt. of lit- ter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
gms.			gms.	gms.	gms.	per cent	
0.3	6/9	10	32	180	13.	-8	
0.2	2/17	8	24	76	5.	+ -0.	One dead at 9 days. Est. of 4
0.6	6/20	6	32	148	11.3	+7.3	
0.6	11/6	8	32	109	7.5	-4.9	
0.6	11/15	9	37	150	10.9	+ -0	Milk Pasteur- ized
0.6	2/18	8	32	141	10.7	+14.2	One dead at 13 days. Est. of 4
0.6	11/19	9	37	109	7.4	-4.9	
0.6	2/19	10	24	105	8.1	-6.6	Milk Past. Second gener- ation
0.6	2/12	8	25	76	4.9	-6.3	Milk pasteur- ized
Average			30.5	121.5	8.7	-1.02	

It will be observed that there was considerable variation in the growth of the young of these milk-fed rats, as well as in the gain or loss in weight of the mothers. There seemed to be little if any relation between gain in weight of the young and loss in weight of the mother. The average gains of the young of mothers receiving the superheated milk preparations (Table II) which, with the exception of dried milk 2, have been shown to contain considerably less antineuritic vitamin than equivalent amounts of quickly boiled milk (14), were higher than those of the young of mothers receiving the simple milk diets (Table I), whereas the average loss of



weight among the mothers receiving the superheated milk preparation was slightly less than those of the mothers on the boiled milk. The average gains of the young of these superheated milk-fed rats were only slightly less than the young of the stock-fed animals (Table IV). The mothers of these stock groups, however, either held their own or made a slight gain during the lactation period.

TABLE IV  
GROWTH OF SUCKLING YOUNG OF RATS FED MILK+ AUTOCLAVED AUTOLYZED YEAST\*

Milk <i>ad lib.</i> + autoclaved au- tolized Yeast. per day	Date	No. born	Weight of litter of 4 at 4 days	Weight of litter of 4 at 22 days	Average gain per rat per week 4 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
gms.			gms.	gms.	gms.	per cent	
0.6	6/7	9	32	130	8.5	+8.3	
0.6	2/19	4	39	150	10.8	+7.	Second genera- tion
0.6	2/22	9	30	172	13.3	-7.	Second genera- tion
0.3	5/21	8	40	170	12.5	-2.	Second genera- tion
0.3	6/28	6	30	128	9.5	+ -0	Second genera- tion
0.3	7/12	7	28	130	9.9	-8.	Second genera- tion
0.3	7/12	6	24	152	12.4	+ -0	Second genera- tion
Average			32	147	11.	+1.7	

\* The autolyzed yeast was dissolved in distilled water and autoclaved at 15 lbs. for 6 hours.

The addition of autolyzed yeast, either autoclaved, or non-autoclaved, was without significant influence on the development of the young, the average in the former group being comparable to that of the superheated milk fed animals, and only slightly less than the stock groups. The young of animals receiving the non-autoclaved, autolyzed yeast concentrate averaged somewhat less than those on the autoclaved, autolyzed yeast. Amongst the mothers receiving the milk and autolyzed yeast, there was considerable variation in the weights during the lactation period. With one exception, in which there was a gain of 14 per cent, these were less marked than those of the superheated or quickly boiled milk groups. The larger amount of autolyzed yeast (0.6 gms. per day), whether autoclaved or non-autoclaved, seemed to protect the mother from the loss in weight

observed among the mothers of the milk and superheated milk groups. The addition of yeast resulted in excellent growth of young in two groups, and fair growth in a third. One mother gained, whereas two lost in weight, one of these more than any other in the series of animals studied.

The young of the animals which received daily 10 cc. of wheat embryo extract in addition to their milk diets gained less than the young whose mothers were receiving the milk only. The additions of copper sulphate, the ash of wheat embryo and of soy beans respectively (Table V) to the

TABLE V  
GROWTH OF SUCKLING YOUNG OF RATS FED MILK+MINERALS

Milk <i>ad lib.</i> + per day	Date	No. born	Wt. of lit- ter of 4 at 4 days	Wt. of lit- ter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
gms.			gms.	gms.	gms.	per cent	
CuSO <sub>4</sub> .0015...	4/28	9	36	168	12.8	-2.	
Wheat embryo. ash .071.....	5/1	9	30	158	12.4	-9.5	
Soy bean ash .071.....	5/8	9	38	130	9.	-8.7	
CuSO <sub>4</sub> .0015...	7/16	7	32	86	5.2	-18.	Second genera- tion
Average....			34	135.5	9.8	-9.5	

milk rations, resulted in fair growth of young and considerable loss in weight (Av.-9.5 per cent) of the mothers. The combination of wheat embryo ash and wheat embryo extract (Table VI) also resulted in satisfactory growth of young, and slight gains in weight of the mothers.

An attempt was made to determine whether the variation in the results with these milk-fed rats could be related to seasonable changes in milk. The experiments, therefore, were grouped according to the month during which the tests were made. Since there seemed to be no evidence that seasonable variations in the antineuritic vitamin content of the milk is the limiting factor in the development of these suckling young, these tables have not been included.

It is quite possible that there are several factors responsible for the less-than-optimum development in the suckling young of some of our milk-fed rats. In the absence of any one, the others would not be manifest; and although the hypothesis of the antineuritic vitamin deficiency cannot

TABLE VI.

## GROWTH OF SUCKLING YOUNG OF RATS FED VARIOUS TYPES OF DIETS

## A. Milk+Wheat Embryo Extract\*

Milk <i>ad lib.</i> + per day	Date	No. born	Wt. of lit- tler of 4 at 4 days	Wt. of lit- ter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 day	Remarks
Wheat embryo extract 10 cc....	2/15	3	gms. 34	gms. 142	gms. 10.5	per cent +3	Second gen. es- timate of 4 on wt. of 3
Wheat embryo extract 10 cc....	2/9	7	28	34	1.17	...	Young died at 13 days
Wheat embryo extract 10 cc....	5/9	8	30	62	4.7	...	Transferred to another diet at 15 da.
Wheat embryo extract 10 cc....	9/17	9	32	116	6.2	+2	Second genera- tion
Average....			29	88.5	5.64	+2.5	

## B. milk+Autoclaved Wheat Embryo Extract\*

Wheat embryo extract auto- claved 10 cc....	3/12	5	28	125	9.4	-2.6	One died at 19 days
---	------	---	----	-----	-----	------	------------------------

## C. Milk—Wheat Embryo\* and Wheat Embryo Ash

10 cc. wheat em- bryo extract + wheat embryo ash .071 gms...	3/5	11	29	166	13.4	+2	Second gen. milk past. Second gen. 2 died on 6th da. milk past. est. 4 on wt. 2 Second gen. milk past.
"	3/24	8	28	144	11.3	+ -0	
"	7/23	8	28	106	7.5	-5	
"	7/31	4	34	140	10.3	+4.8	
"	7/20	5	34	110	7.4	+ -0	
Average....			31	133	9.98	+1.16	

TABLE VI (Continued)  
D. Milk+Dehydrated Yeast

Milk <i>ad lib.</i> + per day	Date	No. born	Wt. of lit- ter of 4 at 4 days	Wt. of lit- ter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
Yeast 0.4 gms...	6/24	7	30	132	9.9	-4.5	
Yeast 0.4 gms...	11/3	8	40	142	13.6	-22.7	
Yeast 0.5 gms...	1/11	6	42	168	12.3	+1.6	
Average....			37	147	11.9	-8.5	

E. Milk+Lettuce

10 gms.....	6/3	6	46	164	13.9	-1.9	
-------------	-----	---	----	-----	------	------	--

F. Stock Ration†

Stock.....	2/12	7	34	158	11.5	+ -0	
Stock+lettuce.	4/23	16	20	170	14.3	+ -0	
Stock+lettuce.	6/7	11	36	176	13.6	+3.8	
Average....			30	168	13.1	+1.2	

† The stock ration consisted of yellow corn, cooked soy beans to which was added 30 grams of  $\text{Ca}_3(\text{PO}_4)_2$  and 15 grams of NaCl to 4 pounds of dry beans, and milk *ad lib.*

\* An 80% alcoholic extraction of wheat embryo so diluted that 1 cc. contains the extract of one gram of the embryo.

be entirely ruled out, our results offer little evidence in support of it. The excellent growth of the suckling young of the animals fed the superheated milks which have been demonstrated to be lower in the antineuritic vitamin than other forms of heat-treated milks, together with the comparatively slight loss in weight of the mothers during the lactation period, suggests that the variations in these milk-fed animals may be due to the idiosyncracies of the rats and not to any antineuritic vitamin deficiency. It may be difficult in some cases to ingest enough milk to quite meet the caloric needs of the lactating animal, or the flavor and texture of the milk may be distasteful. The fact that our animals have never manifested any of the typical symptoms of the antineuritic deficiency also argues against the hypothesis of the vitamin deficiency.

Contrary to the work of Sure (4), we have found in our milk-fed rats no relation between the gain in weight of the young and loss of weight in the mother. Nor have we found in a series of experiments reported elsewhere (14), that a too low antineuritic vitamin content of the diet of the

mother, manifested in the young by the characteristic symptoms outlined, is consistently accompanied by loss of weight of the mother. Among our milk-fed animals there was excellent development of the young in some cases, and outstanding loss of weight in the mother; in others there was excellent development in the young and no loss in weight of the mother.

Sure (15) has postulated that the antineuritic vitamin content of the cow's milk mixtures used in infant feedings may be below the physiological requirement of the human baby. He has based his conclusions on feeding experiments with rats receiving a ration in which the antineuritic vitamin was furnished by 50 per cent of dried milk. Hoobler (16) also has suggested that a milk mixture consisting of cow's milk and corn syrup (Karo) may contain too little vitamin B for the average baby. Indeed it has been observed by a number of investigators (17) that the additions of concentrated extracts of vitamin B preparations to the milk feedings of the artificially fed infant have resulted in stimulation of growth. Our results with rats do not indicate that the antineuritic vitamin content of milk is so low as to need fortifying in the artificial feeding mixtures of normal infants. A suckling rat may double its weight in six days, whereas an infant may double its weight in four to five months. The young rat therefore grows about twenty-five times as fast as the baby. According to Sure, Evans and Burr, and Macy, Outhouse, *et al.*, from three to five times as much of the antineuritic vitamin is required for lactation as for normal growth. In our experiments one rat suckled four young. It would seem, therefore, that any food which can furnish enough of the antineuritic vitamin for the development of four suckling rats, must contain enough for the normal human infant. It would seem, even, that there would be a considerable margin of safety.

In view of the results with our rats, one is led to wonder if the stimulation of growth in infants following the addition of the vitamin B-containing substances to the artificial feeding mixtures was due to other materials contained therein, or to the fact that the antineuritic vitamin in cow's milk is less available to the human infant. Experiments to determine this are in progress.

#### SUMMARY

An attempt has been made to determine if the high mortality of the suckling young of milk fed rats is due to a deficiency of the antineuritic vitamin content of the milk. Stock-fed rats, either just previous to or following parturition, were given various types of milk diets, some of which were distinctly lower in the antineuritic vitamin than others.

The influence of the addition of substances known to be rich in the vitamin was tested, as well as the influence of certain inorganic substances.

The results of the investigation indicate that the less-than-optimum growth in some of the suckling young of the milk fed groups is not due to a deficiency of the antineuritic vitamin in the milk secretion, but to the inability of the particular rat to ingest a sufficient amount of milk to meet its caloric requirements, or to a general distaste for the food so that too little is eaten.

#### BIBLIOGRAPHY

1. Sure, B., *Jour. Biol. Chem.*, 1925, LXII, 371; LXIII, 211.
2. Evans, H. M., and Burr, G. O., *Jour. Biol. Chem.*, 1928, LXXVI, 263.
3. Macy, I. G., Outhouse, J., Long, M. L., and Graham, A., *Jour. Biol. Chem.*, 1927, LXXIII, 153.
4. Sure, B., *Jour. Amer. Med. Assn.*, 1927, LXXXIX, 675.
5. Macy, I. G., Outhouse, J., Graham A., and Long, M. L., *Jour. Biol. Chem.*, 1927, LXXIII, 189.
6. Hopkins, F. G., *Jour. Physiol.*, 1912, XLIV, 425.
7. Kennedy, C., and Dutcher, R. A., *Jour. Biol. Chem.*, 1922, L, 339.
8. Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 1920, XXXIV, 537; 1920, XLI, 515.
9. Johnson, J. M., *Pub. Health Rep.*, U. S. P. H., 1921, XXXVI, 2044.
10. Gibson, R. B., and Concepcion, I., *Philippine Jour. Sci.*, B., 1916, XI, 119.
11. Outhouse, J., Macy, I. G., Brekke, V., and Graham, A., *Jour. Biol. Chem.*, 1927, LXXIII, 203.
12. Sure, B., *Jour. Biol. Chem.*, Loc. cit.
13. Sure, B., *Jour. Biol. Chem.*, 1928, LXXIV, 685.
14. Hartwell, G. A., *Biochem. Jour.*, 1925, XIX, 226; Daniels, A. L., and Brooks, L., *Proc. Soc. Exp. Biol. and Medicine*, 1927, XXV, 161; Daniels, A. L. Giddings; M. L. and Jordan D., *This Journal*, 1929, I, 455.
15. Sure, B., *Amer. Jour. Dis. Child.*, 1928, XXXV, 811.
16. Hoobler, R. B., *Jour. Amer. Med. Assn.*, 1928, XCI, 307.
17. Eddy, W. B., *Jour. Biol. Chem.*, 1920, XLI, 34; Daniels, A. L., and Byfield, A. H., *Amer. Jour. Dis. of Child.*, 1919, XVIII, 547.





## PROTEIN METABOLISM OF CHILDREN ON DIETS EXTREMELY LOW IN CARBOHYDRATES

BY IRVINE MCQUARRIE,

With the Technical Assistance of Mrs. Ruth Turner Sheahen

*(From the Department of Pediatrics, University of Rochester,  
School of Medicine and Dentistry, Rochester, N. Y.)*

Received for Publication—May 18, 1929

The use of diets high in fat and extremely low in carbohydrate in the treatment of idiopathic epilepsy has become increasingly popular in the pediatric clinics of America since its introduction by Wilder (1) and Peterman (2). It was first suggested as a possible substitute for fasting, which had previously been shown by Guelpa and Marie (3) and by Geyelin (4) to cause a cessation of seizures in many severe cases of the disease. The peculiar effectiveness of fasting and of the ketogenic diet was thought by Wilder and Peterman to be due to the anaesthetic action of the ketone bodies formed in the tissues. Other observers believe that it owes its therapeutic value to its effect upon the acid-base equilibrium. The latter believe that it corrects an abnormal tendency toward the spontaneous development of alkalosis in these subjects. While there is some theoretical evidence in favor of each of these more or less plausible explanations, we believe at the present time that the dehydrating effect of such regimens is the primary factor in favoring the cessation of convulsions (5).

No matter what the true mechanism of its action may be, the low-carbohydrate, relatively high-fat diet, together with definite restriction of the total water intake, offers the most effective and most promising type of therapy for the epileptic child. Since the patient must remain on rather limited regimen almost indefinitely, it is extremely important that every precaution be taken to prevent injury to the body from any sort of dietary inadequacy. Not only the total energy value of the diet but its content in protein, vitamins, mineral elements and other necessary constituents must be considered in order that optimum amounts for normal growth and development may be given. The present communication deals chiefly with the phase of the problem relating to the protein requirement.

Practically all of the authors (2,6,7) who have described their practise as regards protein allowance in the strongly ketogenic diets used, have



assumed that one gram of protein per kilogram of body weight is sufficient to meet all requirements. In a carefully conducted study of the protein needs of diabetic children between the ages of 4 and 14 years on relatively high-fat, non-ketogenic diets, Bartlett (8) found that from 0.6 to 1.0 gm. per kilogram of body weight was sufficient to maintain a positive nitrogen balance and to allow normal growth and development, so long as the total caloric requirements were fulfilled. No similar study has been made on children living on the strongly ketogenic diets used in the treatment of epilepsy. In connection with other experimental studies on a series of epileptic children receiving extremely low carbohydrate diets, we have made observations on the nitrogen balance under different conditions. The following is a report of results from six of these subjects.

#### PLAN OF STUDY AND METHODS USED

The time of each experiment was divided into periods according to variations in the dietary regimens which were being tested as to their efficiency in preventing epileptic seizures. With a few exceptions, the total caloric value of the diet remained unchanged throughout individual experiments. The special influence of the acid-forming and base-forming elements of the diet was determined in two instances. The effect of convulsions on the total amount of nitrogen eliminated was incidentally observed.

The patients, ranging in age from 5 to 15 years, were entirely well throughout the periods of observation except for the convulsive seizures, which they had during certain of the sub-periods. They were kept in bed during periods of fasting and for a portion of each day during the special diet periods, but were allowed to walk about their rooms for mild exercise at other times. Water was allowed *ad libitum* in the experiments reported here, but it may be said in passing that the factor of dehydration, as observed in some of our other studies, has exerted no apparent influence upon the nitrogen balance. The patients were managed in accordance with such requirements as those outlined by Gephart and DuBois (9), so far as this is possible in an ordinary pediatric division, and the diets were carefully calculated from the tables of Atwater and Bryant (10). The total caloric values of the diets were calculated to be approximately adequate for maintaining body weight under ordinary conditions or for allowing slight gains only. The proteins used were varied, but were chiefly from animal sources. No experiments have been included in which the food given was not all consumed or in which urine specimens were incomplete. All specimens were kept in the refrigerator with toluol

as a preservative. Determinations were always made daily on the 24-hour samples, excepting on Sundays. The latter exception appears to have made little or no difference under the circumstances. Unfortunately, fecal nitrogen could not be determined regularly, but was determined a few times in patients on different levels of protein intake in order that the approximate magnitude of this factor might be appreciated. In these instances the samples were collected in 4- or 5-day periods marked by carmine or charcoal in the usual manner. The total nitrogen of the urine and of the feces was determined by the macro-Kjeldahl method as modified by Folin and Wright (11). The permutit method of Folin and Bell (12) was used for the urinary ammonia. In order that the relationship of ketosis to protein destruction might be ascertained, the urinary excretion of acetone bodies was determined, the colorimetric method of Behre and Benedict (13) being used. For the total titratable acid of the urine the procedure of Henderson and Palmer (14) was employed.

### RESULTS

For the sake of brevity and clearness, the data obtained are presented in graphic form on the accompanying charts, which are largely self-explanatory. The body-weight curves in the various charts indicate that the energy values of the rations given were sufficiently high to prevent loss in weight beyond the slight temporary changes due to the dehydrating effect of such diets (15).

When it is desirable to consider the fecal nitrogen, it will be necessary to accept approximate estimations based upon a small number of determinations made on children taking maintenance diets, the constituents of which were similar to those in the present study. The approximate ratios of urine nitrogen to fecal nitrogen for three different levels of protein intake in these cases were as follows: for 4 grams of protein per kilogram, of body weight, 13 to 1; for 2 grams per kilogram, 10 to 1 and for 1 gram per kilogram, 10 to 1. It is obvious that these average ratios are modified by too many factors to be of real assistance in accurate calculations of complete nitrogen balance, but they are of value in indicating the approximate amount of nitrogen lost by this route.

The data recorded in Chart 1 were obtained from a 40-day experiment with an epileptic girl 8 years of age on a carbohydrate-free diet. This period was subdivided into five almost equal periods, in which different levels of protein intake were maintained. The diet remained isocaloric throughout the entire time, a sufficient number of calories in the form of fat being added to replace the protein calories withdrawn with each

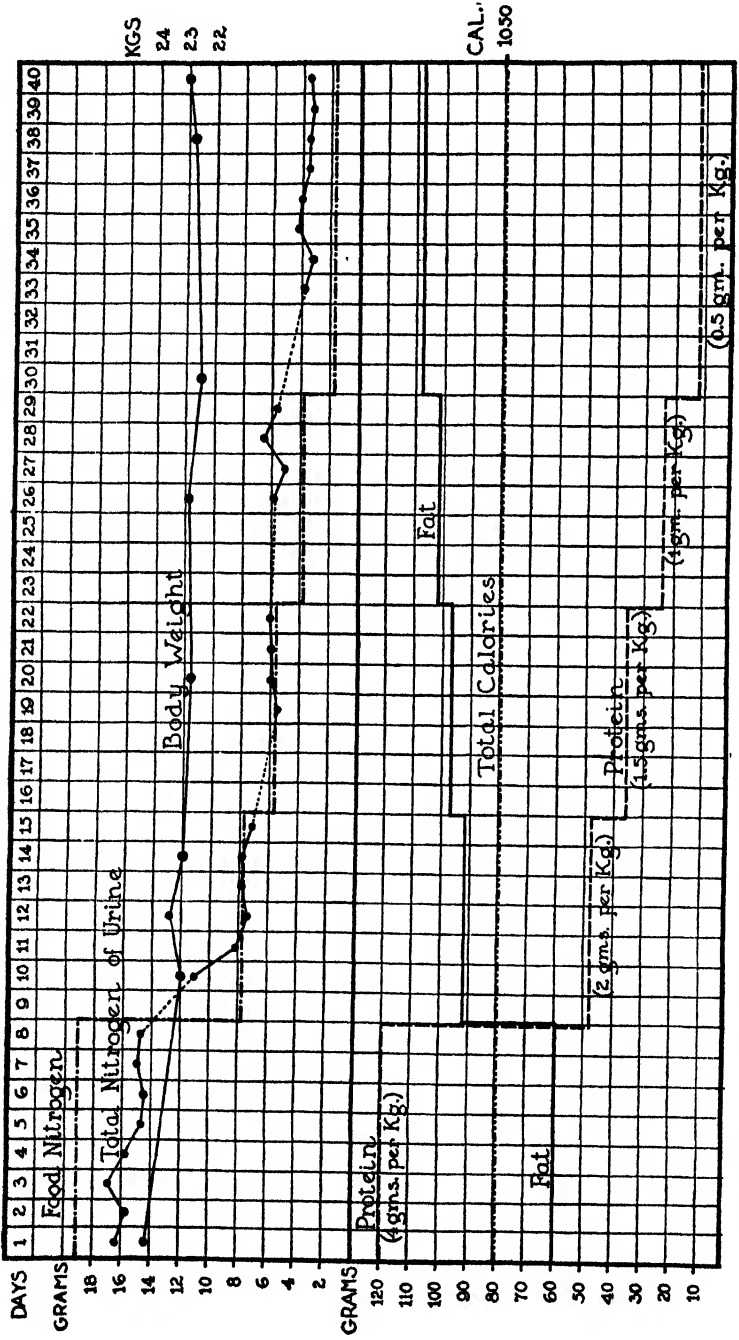


CHART 1. Variations in nitrogen balance on different levels of protein intake. Carbohydrate-free diet. Patient: J. T. epileptic girl, aged 8 years.

change. The initial loss in body weight, amounting to slightly over one kilogram, occurred during the period of very high protein ingestion. There are two characteristics of the diet itself which explain this, namely, its dehydrating effect (15) and the specific dynamic action of the large amount of protein eaten. Assuming the fecal to be one thirteenth as much as the urinary nitrogen, it is apparent from the chart that the patient maintained a positive nitrogen balance in spite of this decrease in weight. During the second period, when the protein intake was 2 grams per kilogram of body weight, the curve representing the quantity of urinary nitrogen practically coincided with that designating the nitrogen intake. An almost identical relationship is seen also between the two in the third period, when 1.5 grams of protein per kilogram were given. There was, therefore, a negative nitrogen balance in these two periods equivalent to the amount of the fecal nitrogen. During the two succeeding periods, when the ratio of ketogenic to ketolytic substances entering into the metabolism was greatly increased, the ratio, food nitrogen: urinary nitrogen, reached a level far below unity. From the data obtained it is estimated that the subject destroyed approximately 14 extra grams of her body protein daily during the last period and 15 grams daily during the preceding period. There were no definite symptoms directly referable to the protein underfeeding during the time of observation.

Chart 2 presents similar data obtained from an experiment on a 13 year old epileptic boy, who had but two mild seizures during the entire period of observation. The diet consisted entirely of protein and fat. Four levels of protein intake, ranging from 4 to 0.67 grams per kilogram of body weight, were tried over periods of 7 days each. There was an initial loss of weight similar to that noted in the previous case, but this was later regained when more fat and less protein were given. It is evident from the chart that the average ratios of food nitrogen to urinary nitrogen for the various periods were not very different from those in the experiment on the younger girl. During the short final period, when 0.67 grams of protein per kilo were given, there was an average daily loss of nitrogen equivalent to 26 grams of body protein.

The comparatively short experiment outlined in Chart 3, is similar to the foregoing, except for the fact that the order of the periods was reversed and the diet contained a small amount of carbohydrate. The subject of the study was a 14 year old boy with moderately severe epilepsy. Inspection of the results reveals the fact that there was a very significant negative nitrogen balance during the initial period when but one gram of protein per kilogram of body weight was taken. During the second

period, however, it is evident that the patient was in nitrogen balance or showed slightly positive balance on 2 grams of protein per kilogram. The food contained 12.8 grams, whereas the urine contained but 11.3 grams daily. There was, therefore, an average of 1.5 grams more nitrogen in the diet than in the urine. If the 10 to 1 ratio of urinary to fecal nitrogen is used to estimate the probable amount of nitrogen lost in the stool,

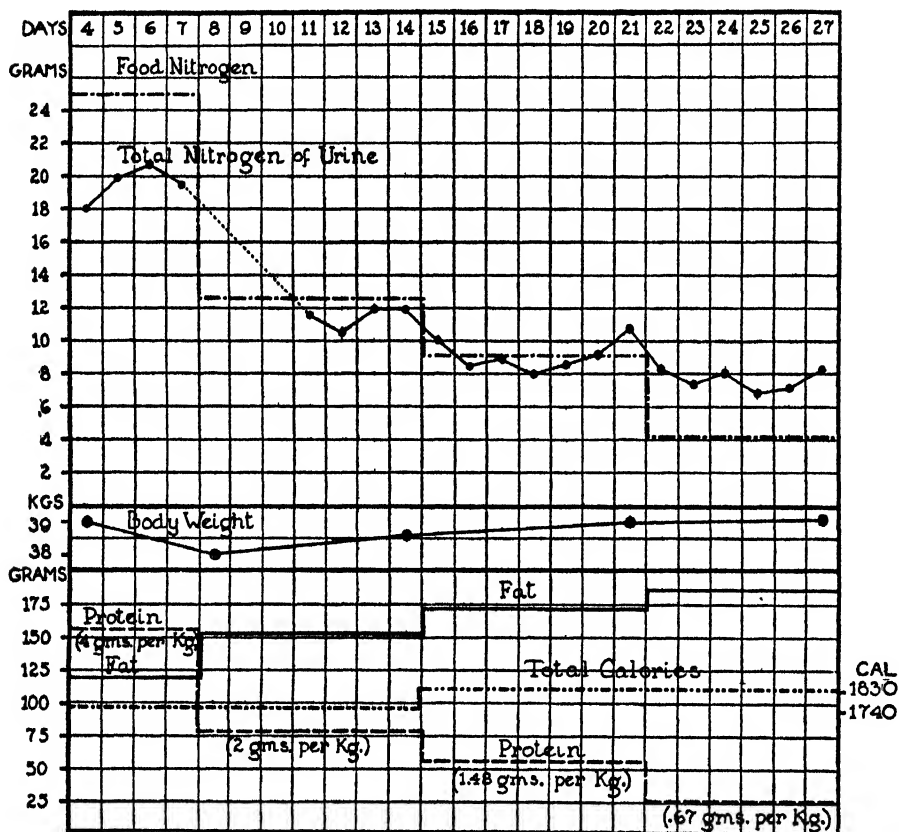


CHART 2. Nitrogen intake and output of J. B., a mildly epileptic boy aged 13 years, on carbohydrate-free diets containing varying amounts of protein.

it is evident that there was an average retention of approximately 0.4 grams daily. For the last period, during which the diet contained 3 grams of protein per kilogram of weight or 19.2 grams of nitrogen daily, the 24-hour output of nitrogen averaged 15.5 grams by way of the kidneys and 1.3 grams (estimated) by way of the intestinal tract. There was thus a retention of nitrogen amounting to approximately 2.4 grams daily. Comparison of these results with those of the previous experiments shows

that even so small an amount of carbohydrate as 15 grams had a very definite protein-sparing effect.

The relationship of the state of ketosis to the nitrogen balance is shown graphically in Chart 4. The data presented were obtained from a study

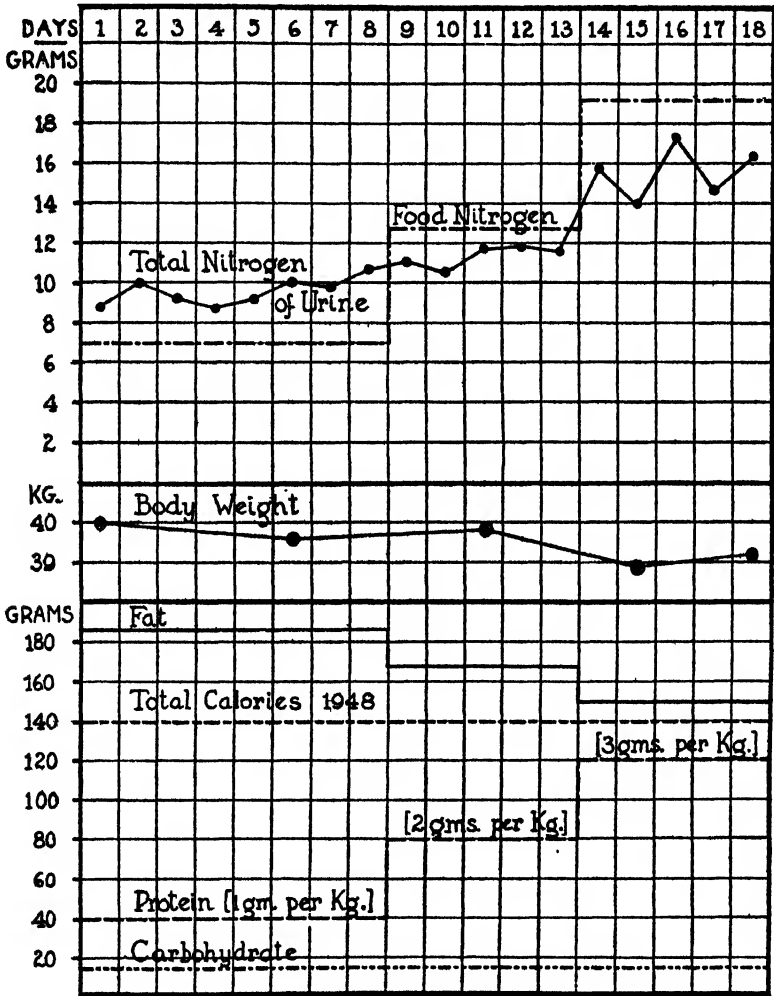


CHART 3. Nitrogen balance study on B. J., an epileptic boy 14 years of age, showing protein-sparing effect of carbohydrate.

made over a period of 34 days on a 15 year old epileptic girl. This differed from the foregoing experiments in that two 3-day fasting periods were included. An interesting fact, which is illustrated here and which we have noted repeatedly is that the degree of ketonuria is diminished tempor-

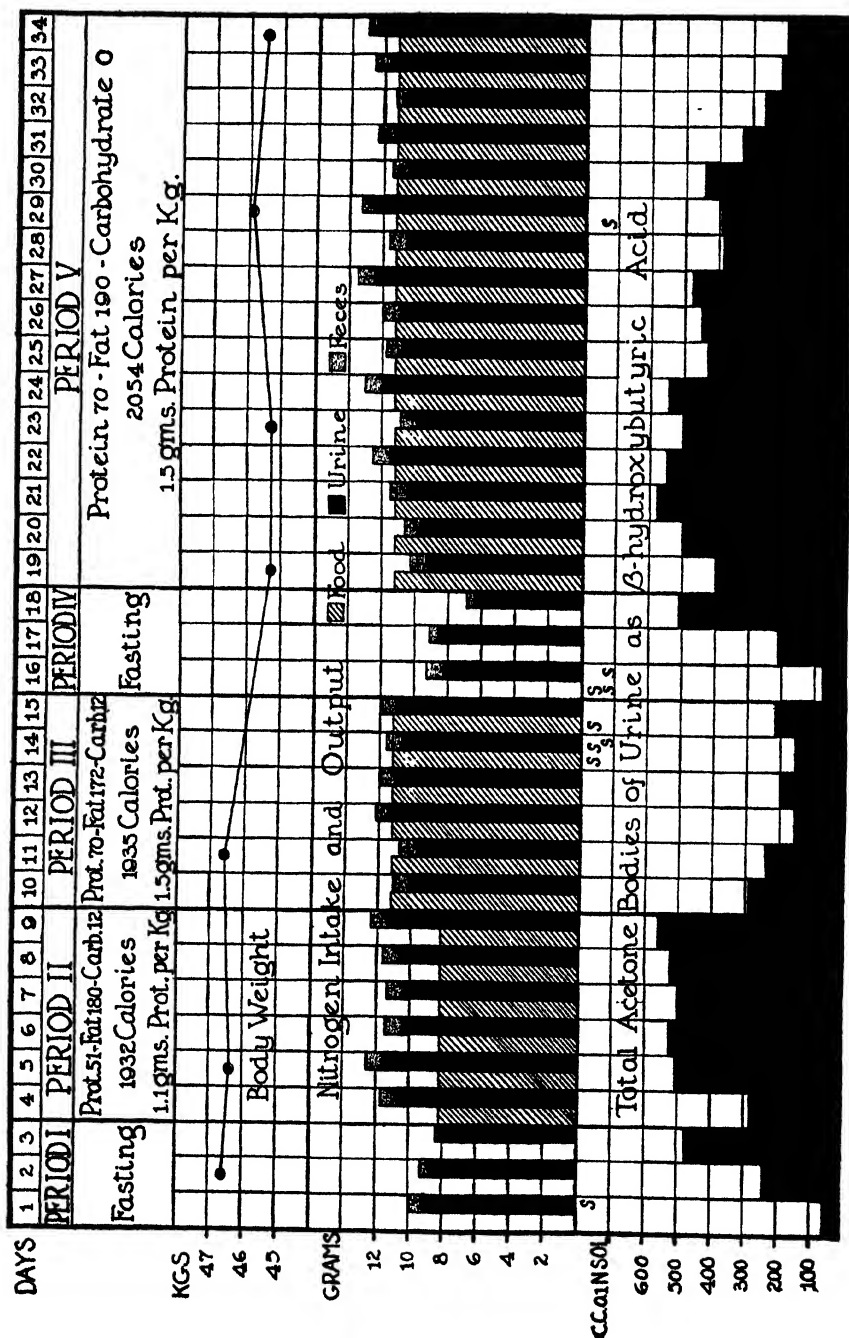


CHART 4. Relationship of fasting and dietary ketosis to protein requirement. J. R., epileptic girl aged 15 years.

arily following the change from fasting to a strongly ketogenic diet or *vice versa*. Weymuller and Schloss (16) also observed the reduction of dietary ketosis by fasting. The explanation, which they suggest for this, is that the proportion of ketolytic material in the mixture metabolized during the early part of the fast is greater than during the preceding period when the large amount of fat was given. At the same time the sharp reduction in metabolism due to the fast tends to spare body fat. Why the intensity of the fasting ketosis is temporarily diminished, as in our experiments, by diets having fatty-acid-to-glucose ratios above 3 to 1 and which later resulted in degrees of ketonuria greater than those occurring during the fast, is not apparent. It is possible that more of the fat from the diet was shunted to the fat depots immediately after the fast periods to replace some of the body fat removed during the fast.

It is evident that the amount of nitrogen eliminated during the periods of fasting diminished in spite of increasing ketosis. Since the patient had previously been on a ketogenic diet with an inadequate protein allowance before each fasting period, this tendency for conservation of body protein may possibly be explained on the assumption that the stores of 'deposit' protein had been partially depleted.

Although the total calories and the amounts of pre-formed carbohydrates remained the same in periods II and III, it is apparent that the average ratio of food nitrogen to total nitrogen excreted for period III (0.97) was very definitely greater than that for period II (0.69), when the degree of ketosis was much more marked. It is obvious that the convulsive seizures which occurred toward the end of period III did not cause a detectable increase in nitrogen elimination.

The diet of period V differed from that of period III in that 18 grams of fat were substituted for the 12 grams of carbohydrate in the latter. The chief purpose of this part of the experiment, as regards the nitrogen metabolism, was to compare the protein-sparing effect of fat to that of carbohydrate under the special conditions present. In spite of the far greater degree of ketosis during period V, the average ratio of food nitrogen to excreta nitrogen for this period (0.94) was barely below that of period III (0.97). Of course, it is obvious from the chart that this average ratio for period V is slightly too high, because there were several days at the beginning of the period during which food nitrogen was evidently being retained to replace nitrogen lost during the preceding fast. The chart, incidentally shows an additional fact of interest as regards non-diabetic dietary ketosis, namely, that there is a tendency for adaptation on the part of the body, such that the degree of ketosis gradually decreases on



a given diet. It is quite apparent, however, that an allowance of 1.5 grams of protein per kilogram of body weight is inadequate in the presence of ketosis, even in older children.

The 23-day experiment represented in Chart 5 was designed primarily to determine the special influence of the non-ketogenic acid-forming and base-forming elements of the ketogenic diet on the seizure tendency in

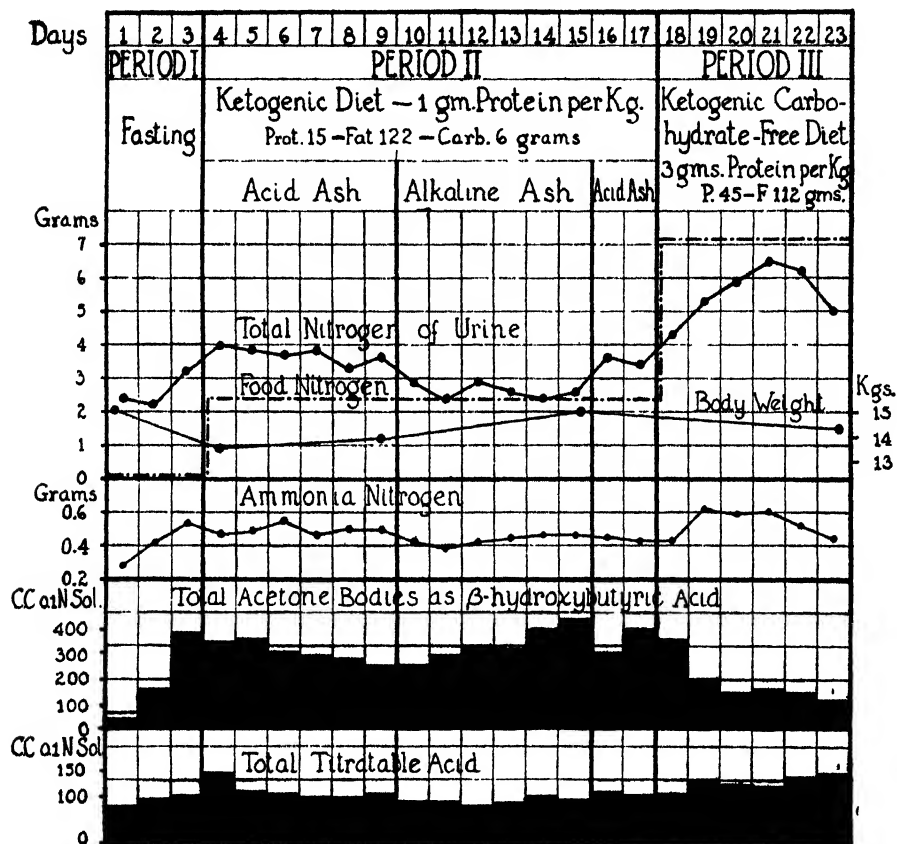


CHART 5. Nitrogen balance during ketosis as influenced by the fixed acid and fixed base content of the diet. S. G. epileptic boy, aged 5 years.

the epileptic patient. Following a three-day preliminary fast, the subject, a 5 year old boy with severe epilepsy, was placed for 14 days on a maintenance ketogenic diet, containing P. 15, F. 122 and C. 6 grams. For the first 6 days the carbohydrate was derived exclusively from the cereal grains, which have an acid ash; while, during the next 6 days, it was given in the form of the leafy vegetables, the ash of which is preponderantly alkaline. Since a considerable amount of bran was included in the diet of

the first 6 days, the amount of indigestible roughage was not very different during the two periods. The base from the base-forming elements in the alkaline-ash was supplemented by the amount of  $\text{NaHCO}_3$  calculated to be necessary for complete neutralization of the excess of inorganic acid derived from the acid-forming constituents. Following the alkaline-ash period, the diet of the acid-ash period was resumed for two days before the third general period was begun. The latter was characterized by complete lack of carbohydrate in the diet, which, however, remained isocaloric with the preceding.

Following the increase in urinary acetone bodies during the fast, there was a significant rise in the total nitrogen of the urine on the last day. The ammonia nitrogen was increased at the same time but is seen to account for a minor fraction only of the excess total nitrogen. The demand made upon the body protein for ammonia to neutralize the extra organic acids formed under these conditions is far less significant than that for the ketolytic fraction of the protein molecule. This is apparently true for dietary as well as for fasting ketosis. Changes in the total titratable acid of the urine do not correspond directly with those in the total acetone bodies. The only significant change was the gradual rise during the last period of the experiment, when the amount of protein in the diet was trebled.

The most interesting feature of the study is brought out by a comparison of the results during the "acid-ash" and "alkaline-ash" sub-periods of period II. Although the diet for both periods contained the same amounts of protein, fat and carbohydrate, the average ratio, food nitrogen : urinary nitrogen, was 0.65 for the former and 0.92 for the latter. The urinary ammonia was slightly higher during the acid-ash period and the total acetone bodies 15 per cent lower than during the alkaline-ash period. Body weight lost during the fast was rapidly regained during the latter period. A goodly portion of this was probably in the form of water. There were many more convulsive seizures during this period than during the acid-ash periods.

It would appear from this experiment that the added base exerted a protein sparing effect. It has been shown by Rubner (17), and confirmed by others, that indigestible roughage in the diet increases the proportion of nitrogen lost by way of the stools. The difference between the amounts of roughage in the two diets under comparison was too small, however, to explain the 30 per cent decrease in urinary nitrogen during the alkaline-ash period. The small difference in the ammonia nitrogen for the two will account for but a minor fraction of the sparing effect observed. Adolph (18) has shown clearly that the rate of elimination of ingested urea and

ammonium salts in the case of a normal subject is dependent in large measure upon the acid-base equilibrium of the body. He found that these substances were excreted by the kidneys more readily when the balance was toward the acid side and less so in the presence of a reaction in the opposite direction. While the conditions of his experiments and those under discussion were somewhat different, it seems probable that the factor of acid-base equilibrium *per se* might have contributed to the difference in nitrogen excretion in these two periods. However, another possible explanation for the greater amount of urinary nitrogen during the acid-ash period is that body protein was less extensively utilized for its ketolytic fraction during the alkaline-ash period. Haldane (19) has shown that alkalosis induced in a normal man by alkali administration inhibits carbohydrate metabolism to a marked degree and produces ketosis. The increase in the intensity of dietary ketosis together with the diminished nitrogen output in our experiment strongly suggests that the process of breaking down tissue protein for its antiketogenic fraction was held in relative abeyance during the alkaline-ash period because carbohydrate metabolism *per se* was less active.

As seen from the chart, positive nitrogen balance was established during period III, when the diet contained 3 grams of protein per kilogram of body weight. The greater degree of nitrogen retention during the early part of the period probably indicates replacement of the body protein lost during the previous periods. The increase in ammonia nitrogen corresponds with the increase in the total titratable acidity of the urine resulting from the increased utilization of protein.

That the extra muscular exertion incident to the convulsive seizures had little or no effect on the total nitrogen balance is again evident from the fact that seizures were far more frequent during the alkaline-ash period and during period III than at other times.

In Chart 6 are presented the results of an experiment on a 13 year old epileptic girl, which extended over a period of 64 days. This time was divided into sub-periods, during which various dietary regimens were tested regarding their efficiency in controlling seizures. The total urinary nitrogen, ammonia, acetone bodies and titratable acidity were determined daily throughout the 64 days. The body weight was measured at frequent intervals. The diet remained isocaloric except for two 4-day fasting periods and the last dietary period. In the latter instance it was increased 60 calories daily.

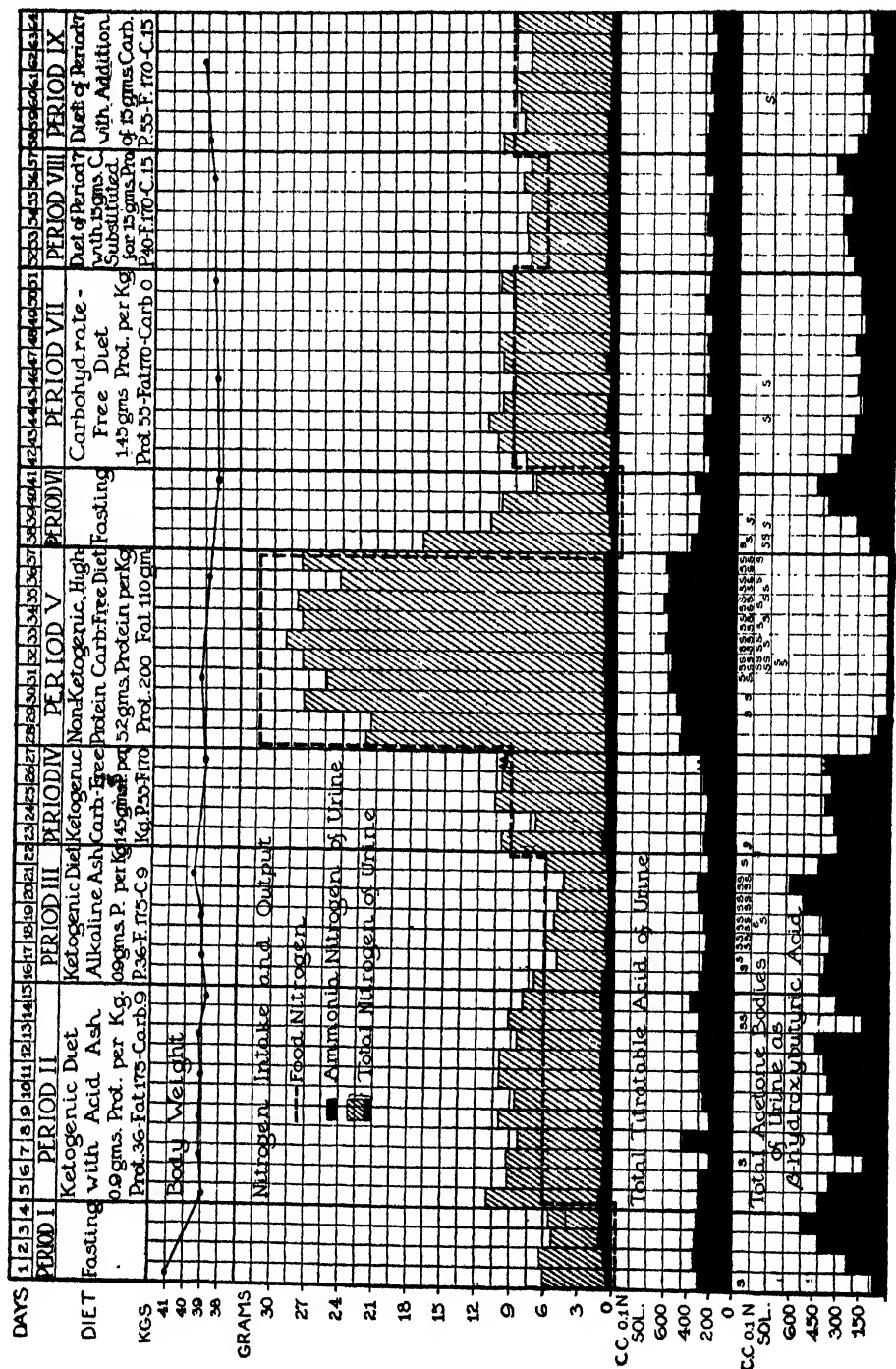
The body weight remained practically uniform when the diet was being given, although there was the expected loss during the fasting periods.

While the degree of ketosis measured for the two fasting periods was practically the same, the nitrogen elimination was very different. In the initial fasting period, which followed a period of comparatively low nitrogen intake, the average daily excretion by way of the kidneys was 5.9 grams; whereas, the average for the second fasting period, which followed an extremely high protein diet, was 11.2 grams.

The data for periods II and III are similar to those of period III of the preceding experiment (see Chart 5). The diets of these two periods were identical, except for the fact that the diet of period III was arranged to have a basic-ash in the manner described in the preceding protocol. The apparent protein-sparing effect of the extra base given during period III is striking when compared with the nitrogen waste of period II. The daily average of the ratio, food nitrogen : urinary nitrogen, for the latter period was 0.66, whereas that for period III was 1.07. The possible reasons for this difference have already been suggested. It is obvious that 0.9 grams per kilogram of body weight is insufficient for complete nitrogen balance in the presence of dietary ketosis. The data of diet period IV show that 1.5 grams protein per kilo of weight is inadequate, when the diet contains no carbohydrate but a preponderance of fat. During period V no carbohydrate was allowed but a sufficiently large proportion of the total calories (over 44 per cent) was given in the form of protein to prevent the development of ketosis. The ketosis existing at the beginning of the period had disappeared entirely by the third day. Although the urinary nitrogen averaged 26 grams per day and the stool nitrogen 2 grams, there was a positive balance of nitrogen, amounting to 6 grams daily. A large portion of the ingested protein was doubtlessly utilized for the antiketogenic portion of its molecule. Another reason for the excessive utilization of protein at this time was the greater demand for energy due to the increased frequency of seizures and to the specific dynamic effect of the protein given. There was probably not only a replacement but an added storage of "deposit" protein during the period, as judged from the excessive output of nitrogen during the first days of the fast that followed.

The diet of period VII was the same as that for period IV and the effects were almost identical, except for the fact that the ketonuria was definitely less marked. It is obvious from a glance at the chart that the average ratio of food nitrogen to urinary nitrogen is slightly less than unity (0.93) as in period IV. In period VIII, there was a substitution of 15 grams of carbohydrate for 15 grams of the protein given in the diet of period VII. Otherwise the two were the same. It is seen that the average ratio, food nitrogen : urinary nitrogen, was lower (0.82) in period VIII, although

CHART 6. Variations in nitrogen metabolism with different levels of protein intake and different degrees of ketosis. Comparison of nitrogen excretion of fasting periods preceded by different levels of protein ingestion. Effect of fixed acid and fixed base of ketogenic diet upon nitrogen balance. M. P. epileptic girl, aged 13 years.



the daily urinary nitrogen averaged more by 2.2 grams during period VII. The results show that the diet of period IX, which was identical with that of period VII except for the addition of 15 grams of carbohydrate, brought the patient nearer to nitrogen balance, the food-nitrogen to urinary-nitrogen ratio being 1.08. Of course, if the amount of fecal nitrogen is estimated to be 10 per cent as great as that of the urine, it becomes obvious that there was a small negative nitrogen balance for the period.

### DISCUSSION

The data presented show very definitely that up to the present time far too little protein has been allowed in the strongly ketogenic diets used in the treatment of epilepsy in children. In the present experiments the attempt was made to determine the approximate amounts required for nitrogen balance under different conditions, no effort having been made to determine the extra amount needed for normal growth of body tissues. It is certain that the ketosis, for which these diets have in the past been given, steps up the protein requirement very greatly. We feel certain that two grams per kilogram of body weight is much nearer the true protein requirement in the very strongly ketogenic regimens than the one gram previously considered adequate.

While we have no definite evidence of harmful effects from our comparatively short-time experiments, it is practically certain that such effects would manifest themselves after many months on such a regimen. We have recently found evidence of a close relationship between the water balance of the body and the occurrence of seizures in epilepsy (5). It is entirely possible that certain patients, who have become refractory to the ketogenic diet, after adhering to it over a considerable period of time, have done so because of a tendency toward abnormal hydration of the tissues secondary to partial protein starvation. The ultimate effect of a diet deficient in protein upon growth and development is too well known to require special comment here.

Whether the protein-sparing effect of added base during dietary ketosis, as demonstrated in experiments 5 and 6 (Charts 5 and 6), might be desirable in some situations is possibly open to question, but its employment in the case of the epileptic subject is rendered impossible by the fact that alkali administration tends to increase the frequency of seizures. The amount of protein required to establish positive nitrogen balance and allow an increase in growth without carbohydrate and with too little fat to permit the development of ketosis, is extremely great, but this can be done. From the observations of Stefansson, regarding the Eskimos, and

of Lieb and Tolstoi (20) on human subjects living exclusively on meat diets, it appears to be certain that no harm is done by diets extremely low in carbohydrate, even when these are used over long periods of time. We have observed that the Eskimo type of diet, even when non-ketogenic, is often effective in preventing epileptic seizures, if the water intake is rather rigidly limited.

Another interesting and probably significant observation, regarding the effect of the low-carbohydrate type of diet upon the general condition of children, is that they appear to remain comparatively free from the common acute infections and complain less of the cold weather during the winter months. It is planned to make some properly controlled studies on these and other similar questions within the near future.

#### SUMMARY AND CONCLUSIONS

The extra factors governing the protein requirement of epileptic children on diets high in fat and extremely low in carbohydrate have been studied in six subjects, ranging in age from 5 years to 15 years. It has been demonstrated clearly that the protein allowance, now being used rather widely, of one gram per kilogram of body weight is far too meagre for growing children on strongly ketogenic diets. When as much as one-half to one-third of a gram of carbohydrate per kilogram of weight is being given, one and three-fourths to two grams of protein is probably adequate. More should be given when the carbohydrate intake is lower than this.

The factor chiefly responsible for the excessive demand on the protein under these conditions is the ketosis, which taxes the protein molecule for its ketolytic fraction. When sufficient base occurs in the diet to approximately neutralize the acid from its acid-forming constituents, the rate of nitrogen excretion is definitely decreased. Possible explanations for this are discussed.

The epileptic convulsion *per se* appears to exert very little, if any, direct effect upon the total urinary nitrogen. Possible additional uses for diets low in carbohydrate and high in fat and protein are mentioned.

1. Wilder, R. M., *Mayo Clinic Bull.*, 1921, II, 307.
2. Peterman, M. G., *Amer. Jour. Dis. Child.*, 1924, XXVIII, 28.
3. Guelpa, G., and Marie, A., *Rev. de therap. Med.—chir.*, 1911, LXXVIII, 8.
4. Geyelin, H. R., *M. Rec.*, 1921, XIXC, 1037.
5. McQuarrie, I., and Husted, C., *Amer. Jour. Dis. Child.*, 1929 (In press).
6. Talbot, F. B., Metcalf, K., and Moriarty, M., *Amer. Jour. Dis. Child.*, 1926, XXXII, 316.
7. McQuarrie, I., and Keith, H. M., *Amer. Jour. Dis. Child.*, 1927, XXXIV, 1013.
8. Bartlett, W. M., *Amer. Jour. Dis. Child.*, 1926, XXXII, 641.

9. Gephart, F. C., and Du Bois, E. F., *Arch. Int. Med.*, 1915, XV, 829.
10. Atwater, W. O., and Bryant, A. P., *Bull. No. 28 (Revised Edition)*, United States Bureau of Agriculture 1906.
11. Folin, O., and Wright, L. E., *Jour. Biol. Chem.*, 1919, XXXVIII, 461.
12. Folin, O., and Bell, H. D., *Jour. Biol. Chem.*, 1917, XXIX, 329.
13. Behre, J. A., and Benedict, S. R., *Jour. Biol. Chem.*, 1926, LXX, 487.
14. Henderson, L. J., and Palmer, W. W., *Jour. Biol. Chem.*, 1914, XVII, 305.
15. See Lusk, G., *The Science of Nutrition*. 4th Edition, 1928. pp. 354-355. W. B. Saunders Co. Philadelphia and London.
16. Weymuller, C. A., and Schloss, O. M., *Amer. Jour. Dis. Child.*, 1927, XXXIV, 549.
17. Rubner, M., *Arch. f. Physiol.*, 1916, 40.
18. Adolph, A. E., *Amer. Jour. Physiol.*, 1925, LXXI, 355.
19. Haldane, J. B. S., *The Lancet*, 1924, I, 537.
20. Lieb, C. W., and Tolstoi, E., *Proc. Soc. Exper. Biol. and Med.*, 1929, XXVI, 324.

We wish to express our gratitude to Miss Clara Husted for making the illustrative charts for this report.







## THE VITAMIN B AND THE VITAMIN G REQUIREMENTS OF THE ALBINO MOUSE

BY FRANKLIN C. BING AND LAFAYETTE B. MENDEL

*(From the Laboratory of Physiological Chemistry, Yale University,  
New Haven, Connecticut)*

Received for Publication—May 25, 1929

THE fact that vitamin B, old nomenclature (1), consisted of at least two factors, one of which was more heat-stable than the other, was perhaps first clearly demonstrated by testing one vitamin preparation on two species of animals. Thus, Smith and Hendrick (2) reported that an amount of a "vitamin picrate" from yeast that Seidell had found sufficient to prevent the loss in weight of adult pigeons fed solely on polished rice, did not permit the growth of young rats. These rats grew, however, when the "vitamin picrate" was supplemented with a small quantity of autoclaved yeast, a material that Goldberger and his collaborators (3) had demonstrated to be ineffective, even in very large quantities, as a substitute for dried yeast in the diet. Numerous observations in other laboratories have fully confirmed and amplified these early demonstrations of the marked difference in stability towards heat of the two factors that were formerly called vitamin B, not only with the rat as experimental animal but with other species as well. The studies of Hauge and Carrick (4) and of Hogan and Hunter (5) indicated that growing pigeons and chickens may require both the heat-stable and heat-labile factors. It seemed desirable to extend such observations concerning the requirements for these factors, vitamins B and G according to present nomenclature (6), to still another species. We have, accordingly, tested various dietary régimes upon the albino mouse. This species although it is very closely related to the rat, has exhibited certain striking differences in its nutritive requirements; some of them have been described by Beard (7).

In the main, our experiments have indicated that the mouse is no exception to the rule that the heat-stable (G) and the heat-labile (B) vitamins are necessary for normal growth. It has seemed from the investigations reported herein that the mouse requires less of the heat-labile fraction, in absolute quantities, than the rat, a finding that is not without interest since Beard has shown (8) that the growing mouse needs just about the same amount of vitamin B (B+G) as a growing rat. Furthermore, the appearance of certain skin lesions somewhat similar to those described by Goldberger and Lillie (9) as the result of a vitamin G de-

ficient diet, has been obtained more consistently, perhaps, than records in the literature would indicate to have been observed in the rat.

### EXPERIMENTS

In these experiments we have used the special inbred strain of *Bagg* albino mouse obtained from the Cold Spring Harbor Station for Experimental Evolution through the kindness of Doctor E. C. MacDowell. Some of the young mice were obtained directly from Doctor MacDowell's laboratory and the rest were bred and reared in New Haven from his original stock. Thus, we were assured of the genetic homogeneity of our experimental animals, a matter of considerable importance in mouse work, we believe, since different strains may vary in their growth and behavior on experimental rations. Young mice between three and four weeks of age were taken from their mothers and placed in individual metal cages with wire-screen false bottoms. Because of the susceptibility of mice to sudden changes in temperature, the animal room was maintained between 74° and 80° F. during the entire time the experiments were in progress.

The basal diet consisted of purified casein 31 per cent, purified cornstarch 38 per cent, hydrogenated vegetable oil<sup>1</sup> 24 per cent, and Osborne and Mendel salt mixture<sup>2</sup> 7 per cent. This food, together with drinking water, was available to the mice at all times. In addition the following supplements were fed apart from the rest of the diet. Two drops of cod-liver oil were fed to each mouse daily. One group of mice received daily 200 mgms. of dried yeast<sup>3</sup> per mouse. Another group received 200 mgms. of autoclaved yeast per mouse to furnish the heat-stable factor only. The third group received daily an addition of an alcoholic extract of rice polishings (tiki-tiki) to furnish the heat-labile factor only. The fourth group received daily per mouse 200 mgms. of autoclaved yeast plus two drops, equivalent to two grams of rice polishings, of the undiluted tiki-tiki extract.

The casein was purified according to the directions of Sherman and MacArthur (10) using 60 per cent by weight of alcohol for extraction. This may not have removed the last traces of vitamins B and G from the casein, but it furnished a product of well-defined purity. The cornstarch was also washed with water and extracted with 60 per cent alcohol by weight. The autoclaved yeast preparation was made from the dried yeast by heating in shallow pyrex dishes at 15 pounds gauge pressure for four hours.

<sup>1</sup> The commercial brand "Crisco" was used.

<sup>2</sup> *Jour. Biol. Chem.* xxxvii, 572, (1919).

<sup>3</sup> The product from the Northwestern Yeast Co. was used.

Treatment somewhat similar is known to destroy all of the heat-labile factor, vitamin B, but to leave a considerable portion of the heat-stable factor, vitamin G, unaltered. The vitamin B preparation was made from rice polishings<sup>4</sup> according to the method of Wells (11) for preparing the so-called tiki-tiki extract, using 25 per cent alcohol by weight for the extraction. From two kilos of rice polishings a little over 100 cc. of the sirup were obtained. Evans and Burr (12) have shown that this material is a very potent source of vitamin B but contains very little or none of the G factor.

Our own tests confirm this statement. A group of rats, 21 days old, was placed on a purified diet devoid of vitamin B but containing ample vitamin G as supplied by 200 mgms. of autoclaved yeast daily. The materials used were the same as those employed in the mouse experiments. All of the rats declined in weight and three developed polyneuritic symptoms within four weeks. The food intake decreased during this time but in no case had each animal ceased eating its portion of autoclaved yeast and some of the basal diet when tested in the assay of the tiki-tiki extract. Graded doses of the tiki-tiki extract were given; for this purpose the extract was diluted with 1.5 parts of water so that one drop of the resulting preparation was equivalent to .02 cc. of the original sirup or to .4 grams of the rice polishings. The gain in weight resulting when varying amounts of this source of vitamin B were fed is shown in Table I. Even one drop of the diluted material stopped the decline in weight of vitamin B-

TABLE I

Tiki-tiki extract fed daily in terms of			Average gain per rat in ten days grams
Diluted, drops	Undiluted, cc.	Rice polishings, grams	
1	.02	0.4	8
2	.04	0.8	12
4	.08	1.6	39
8	.16	3.2	50

deficient rats and resulted in a slight improvement in a ten-day period. Four drops or .08 cc. gave good growth and eight drops or .16 cc., very good growth. One rat without any autoclaved yeast or other known source of vitamin G showed but slight retardation of loss of weight even when .20 cc. of tiki-tiki extract was fed daily. From these results we may conclude that the tiki-tiki extract used was a potent source of the B factor and contained very little of the G factor, and the rat required at least .08 cc. of this material daily.

<sup>4</sup> This material was secured from the Louisiana Rice Co. of New Orleans.

## RESULTS

The results of the mouse feeding experiments are given in the growth curves in charts 1 and 2. With autoclaved yeast alone, all of the animals showed a slight initial gain in weight and then a gradual decline followed by death in 21 days. The mice that received two drops of tiki-tiki extract

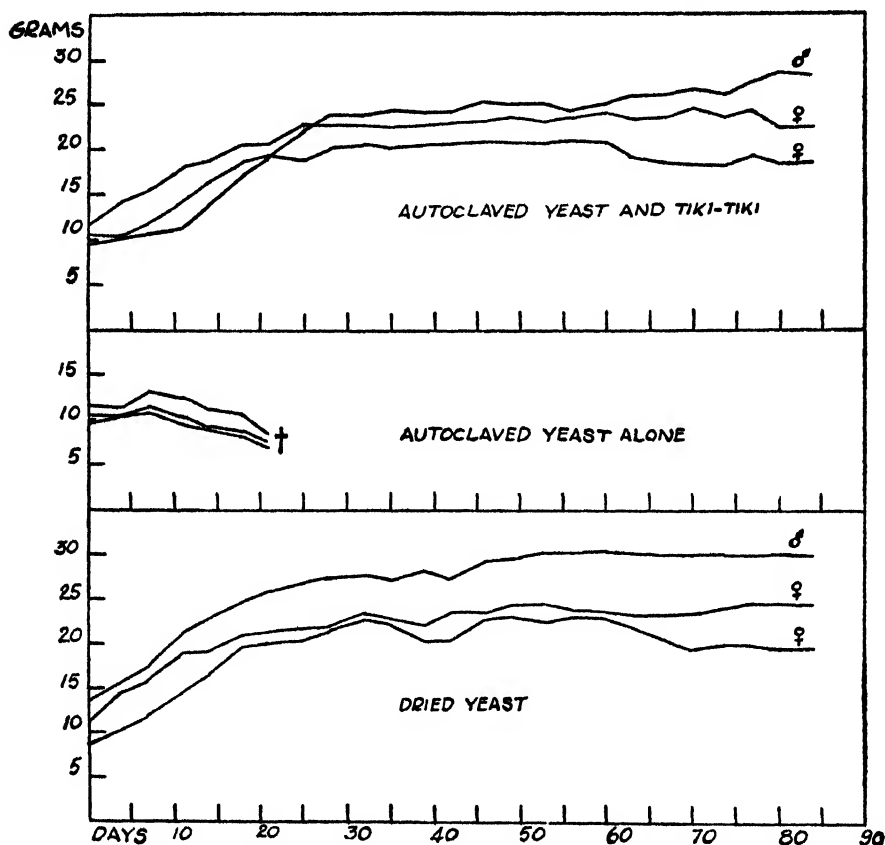


CHART 1. Growth curves of the animals receiving vitamins B (F or B<sub>1</sub>) and G(B<sub>3</sub> or P-P) as supplied by autoclaved yeast and tiki-tiki extract, or as dried and untreated yeast. Growth curves of the animals receiving vitamin G only, as supplied by autoclaved yeast.

daily in addition to the autoclaved yeast grew at rates comparable to the group that received untreated yeast. The growth in both cases may be considered normal.

The behavior of the mice that received the tiki-tiki extract only is illustrated in Chart 2. Two drops of the sirup were fed for three weeks during which time the animals did not grow. The animals then showed a

tendency to grow slightly. The tiki-tiki extract was cut down progressively over a period of two weeks to three drops of diluted tiki-tiki, equivalent to .06 cc. of the original extract, every other day. This amount was just sufficient to enable the animals to maintain their weights.

The question of how much of the tiki-tiki extract the mouse required for normal growth, with the diet otherwise adequate, was ascertained by feeding a second group of mice on the basal mouse diet plus daily additions of 200 mgms. of autoclaved yeast and two drops of cod liver oil. No trouble was experienced in getting the animals to eat the autoclaved yeast. Measurements of the food and water intakes showed that these mice ate diminishingly less food and water on the B-deficient diet, during a period of two weeks. At the end of this period none of the mice was eating more than 0.5 grams of food daily. They were then given supplements of graded doses of the tiki-tiki extract. All the animals regained their appetites at once and in three days the average daily food intake was 5.0 grams per mouse. From the results on Chart 3 it may be concluded that .03 to .04 cc. was enough for good growth. Mouse 35 was in poor condition when given the tiki-tiki extract and did not respond as well as the others. Only one mouse, number 33, in this group showed symptoms of polyneuritis, and they disappeared promptly when the vitamin B was given. Throughout these experiments we endeavored to maintain the same conditions as held with the rat assay of tiki-tiki; the materials used in both diets were the same and the experiments ran concurrently. The amount of the tiki-tiki extract required for a growing mouse was only half, or less, as much as a growing rat required. It has been shown (8) that the mouse requires about the same amount of a vitamin B (B+G) preparation as a young rat, or four times as much per unit weight. Although further experiments must be made before any definite conclusions can be drawn about the amounts of each factor needed for normal growth of rats and mice, these few experiments point out the desirability of extending the present quantitative data to a consideration of both the heat-stable and heat-labile factors.

The supplementary action of autoclaved yeast and the tiki-tiki extract is well illustrated by the growth curves of two mice on Chart 2. Mouse 11 received the tiki-tiki extract only for 35 days, during which time it showed no appreciable loss or gain in weight. Then 200 mgms. of autoclaved yeast were added daily for one week. In this time the animal gained in weight and improved markedly in appearance. The tiki-tiki extract was now removed but the autoclaved yeast continued. The growth curve

showed a sharp drop. The animal lost weight rapidly; in 18 days from 18.1 grams to 9.7 grams. Finally, when the tiki-tiki extract was substituted for the autoclaved yeast the mouse improved somewhat and maintained its weight.

Mouse 6 received autoclaved yeast only for 21 days and at the end of this time it was very weak and felt cold. It did not exhibit any of the

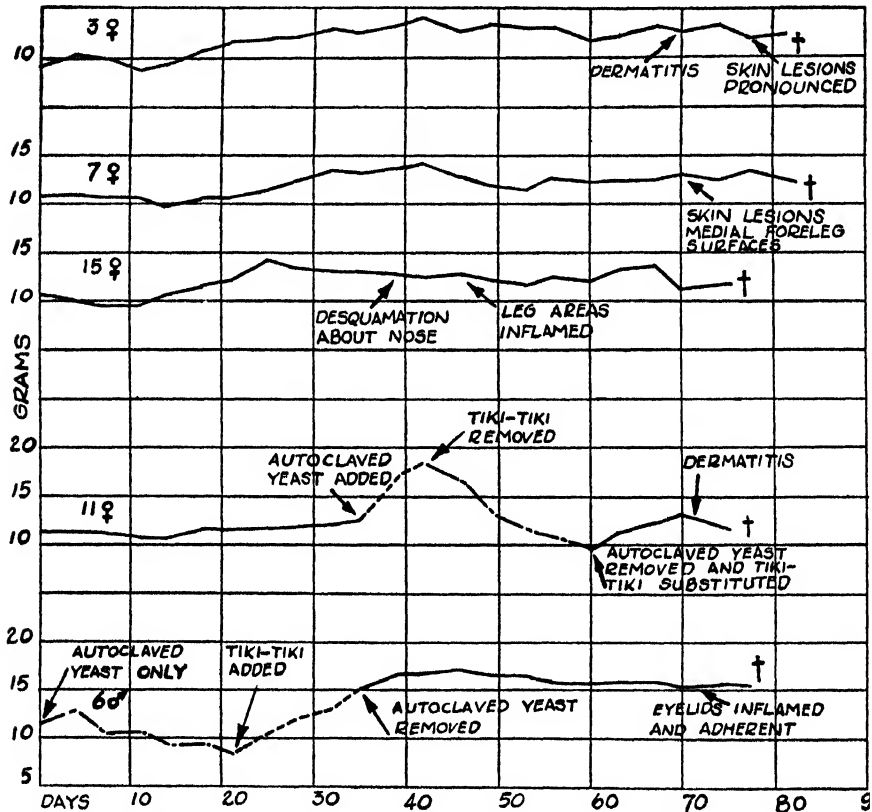


CHART 2. Growth curves of the animals on a vitamin G(B<sub>2</sub> or P-P)-deficient diet, but receiving vitamin B(F or B<sub>1</sub>) as supplied by tiki-tiki extract. The lower two curves are of animals that were fed some autoclaved yeast at the times marked on the graph. The arrows indicate also the time when skin disturbances were noticed on each animal.

nervous manifestations that occasional animals show with a similar diet. Two drops of tiki-tiki extract were given by mouth. The animal was alive the next day and the oral administration of the vitamin B preparation was repeated. Thereafter one drop of diluted tiki-tiki extract, equivalent to .02 cc. of the original sirup, was given daily for 15 days. In this time the mouse increased in weight from 8.2 grams to 15.0 grams and improved

markedly in condition. Upon removal of the autoclaved yeast from the diet, growth ceased and the mouse just maintained its weight.

It was observed that the mice receiving the diet deficient in vitamin G, although they did not grow, lived for a considerable time. These mice showed no loss in appetite and consumed their food and water up to the last two or three days of the experiment. This behavior is in agreement

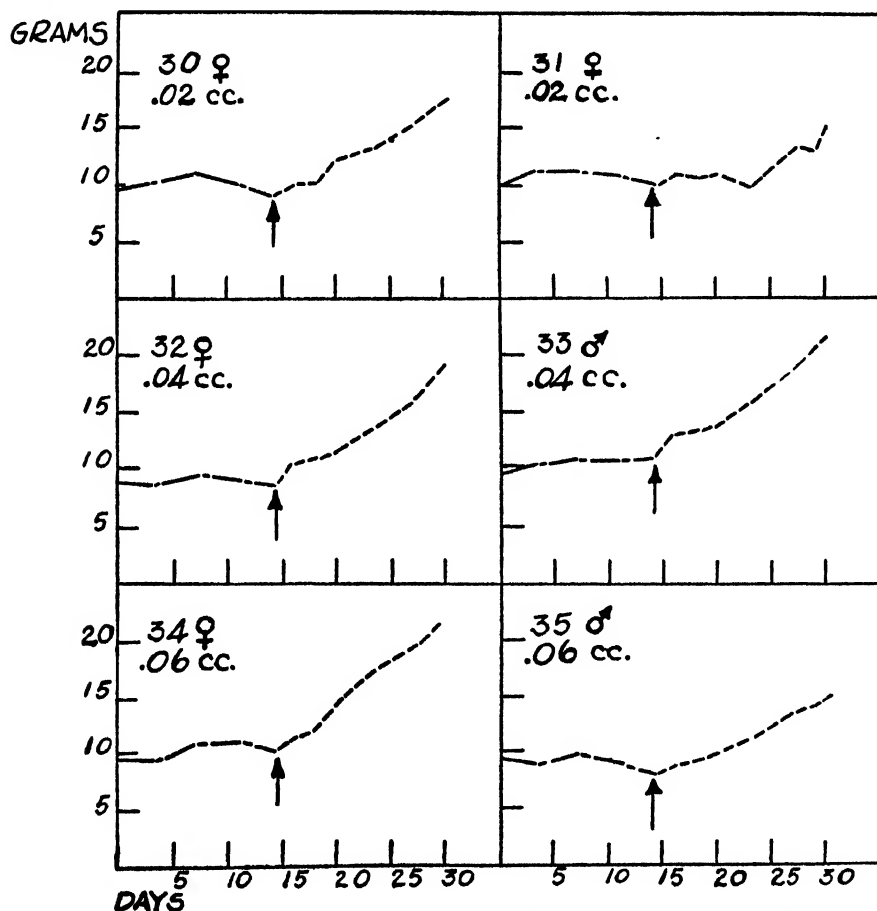


CHART 3. Mouse assay of tiki-tiki extract. The mice received the basal mouse diet plus daily additions of 200 mgms. of autoclaved yeast, and at the point marked by the arrow in each graph, received supplements of graded doses of the tiki-tiki extract as indicated.

with the observations of numerous investigators upon rats. Whether the survival was due to the presence of a small residual amount of the heat-stable factor in the basal diet is difficult to determine. After 39 days on the diet one mouse exhibited signs of skin lesions over the nose. These were first attributed to the animals rubbing against the wire screen of



its cage. However, the skin over the medial surfaces of its hind legs soon became inflamed and other lesions were also observed. The other animals became similarly affected but not until after 70 days on this diet; as is indicated in Chart 2 all the animals on the vitamin G-deficient diet had the skin lesions.

The skin manifestations were very similar to those described by Goldberger and Lillie (9) and Chick and Roscoe (13) with rats on a G-deficient diet. Early in the experiment the fur of these mice had an unkempt appearance but this was no more marked than the derangement of the fur usually seen with almost any deficient diet. Later, the fur became much matted, even though the animals were frequently observed to lick their fur assiduously. The skin beneath assumed a bluish cast; this was particularly noticeable on the abdomen. The first evidence of definite lesions appeared as a slight swelling and inflammation that involved definite areas, usually on the hind legs just over the ankle. In very nearly every case this dermatitis was bilaterally symmetrical. In two of the animals the skin over the surface of the nose was involved, and four had skin lesions under the lower jaw. About four days after the first appearance of inflammation the skin appeared moist from an exudation of serum and the fur began to slough away. A thick yellowish mass of dried serum remained. In the two animals having lesions on the nose, the incrustated mass extended back as far as the eyes, and in one animal involved the eyelids so that these adhered together. Lesions were found on every one of the animals on a G-deficient diet, the most frequent loci being the medial surfaces of both fore and hind legs. Small lesions were sometimes found on the ears, or along the tail, and on the paws. In no case was any healing of the affected regions observed, possibly because the animals did not live very long after the skin disturbances made their initial appearance.

Figure 4 shows the characteristic position assumed by the mice after several weeks on the G-deficient diet. Their hind legs spread wide apart, they squatted with their fore-paws clenched up under them. Frequently the tail was bent back over the body. The exaggerated humped-back and the general appearance of the animals is well illustrated. In no case were any nervous symptoms observed in this group of animals.

When these animals died, immediate autopsy was made, and portions of the skin that showed the lesions were fixed in Zenker's solution and examined histologically. In general the sections from the skin showed the pathology of the later stages detailed by Findlay (14). The epidermis was sloughed away in many places and the exposed derma frequently

showed small ulcerations and infiltrations of polymorphonuclear leucocytes. Although a microscopic study was not made we were not able to observe any congestion or ulceration about the tongue or buccal cavity. The stomach of one animal contained a large hair ball. The intestines of two animals were filled with blood, and the bone marrow of all appeared hemorrhagic. It is interesting to note that the skin lesions on two animals, 6 and 11, developed within a comparatively short time after removal of



FIG. 4—Mouse 3, shortly after death as the result of a vitamin G-deficient diet, in a position that is characteristic of that assumed by the animals after several weeks on the diet. Note the appearance of the fur, the arched back, and the dermatitis on the medial aspect of the right hind leg. Lesions are also present on the corresponding surface of the left hind leg, and on the ventral surface of the skin over the neck.

autoclaved yeast from the diet. This fact may be evidence in favor of the opinion of Sherman and Sandels (15) that the skin lesions develop only when there is some of the heat-stable factor in the diet, complete absence of the G factor probably resulting in early death.

The uniformity of behavior of the individual animals on each diet and the general agreement with the results of rat experiments suggests the further use of mice for investigations of this character.

## CONCLUSIONS

The mouse requires both the heat-stable vitamin G (B<sub>2</sub> or P-P) and the heat-labile vitamin B (F or B<sub>1</sub>) in its diet for normal growth and nutrition. In the absence of vitamin B death occurs in about three weeks. In the absence of vitamin G growth does not proceed, but the mice may live for a considerable length of time. This survival may be due to the presence of small amounts of vitamin G in the vitamin B preparation used or to minute residues in the purified basal rations. Skin lesions, similar to those produced in the rat and termed pellagra-like, have been produced in mice on a diet devoid of vitamin G. The mouse required about half as much of the heat-labile vitamin B as the rat.

## BIBLIOGRAPHY

1. Drummond, J. C., *Biochem. Jour.*, 1920, XIV, 660
2. Smith, M. L., and Hendrick, E. G., *Pub. Health Rep.*, 1926, XLI, 201
3. Goldberger, J., Wheeler, G. A., Lillie, R. D., and Rogers, L. M., *Pub. Health Rep.*, 1926, XLI, 297.
4. Hauge, S. M., and Carriek, C. W., *Jour. Biol. Chem.*, 1926, LXIX, 403
5. Hogan, A. G., and Hunter, J. E., *Jour. Biol. Chem.*, 1928, LXXVIII, 433
6. Dutcher, R. A., *Science*, 1929, LXIX, 276.
7. Beard, H. H., *Amer. Jour. Physiol.*, 1926, LXXV, 645
8. Beard, H. H., *Amer. Jour. Physiol.*, 1926, LXXV, 668.
9. Goldberger, J., and Lillie, R. D., *Pub. Health Rep.*, 1926, XLI, 1025
10. Sherman, H. C., and MacArthur, E. H., *Jour. Biol. Chem.*, 1927, LXXIV, 107
11. Wells, A. H., *Philip. Jour. Science*, 1921, XIX, 67
12. Evans, H. M., and Burt, G. O., *Jour. Biol. Chem.*, 1928, LXXVII, 231
13. Chick, H., and Roscoe, M. H., *Biochem. Jour.*, 1928, XXII, 790
14. Findlay, G. M., *Jour. Path. Bact.*, 1928, XXXI, 353.
15. Sherman, H. C., and Sandels, M. R., *Proc. Soc. Exp. Biol. & Med.*, 1929, XXVI, 536



## AN APPLICATION OF SOME OF THE MORE RECENT METHODS OF ESTIMATING VITAMIN D

BY V. G. HELLER AND CHARLES CASKEY

*(From the Department of Agricultural Chemistry Research, Oklahoma Agricultural and Mechanical College, Stillwater)*

Received for Publication—June 1, 1929

THE line test of McCollum and coworkers, and the methods making use of the chemical analysis of the bone, have been widely used and accepted in the quantitative tests for vitamin D. Recent literature has called attention to certain disadvantages and has suggested new procedures.

One method by Jepchott and Bacharach (1928) makes use of the changing hydrogen ion concentration of the feces, while the other by Poulsson (1928) recommends the use of the X-ray picture in following the rate of decalcification or recalcification. The principles upon which these methods are founded have been described in detail by their respective investigators, and a very complete bibliography covering the entire field has been presented in the recent article by Oser (1928).

A careful study has been made of the application of these methods and certain changes in technique have been suggested. The purpose of this paper is to outline a rapid method of procedure not given in detail in the original articles. A detailed method for the use of the quinhydrone electrode in the  $pH$  determination has been developed.

### EXPERIMENTAL

Albino rats from our own colony have been used as test animals. The colony ration for several years has consisted of a mixture of grains, supplemented with milk powder and minerals. The experimental animals were chosen from stock, 30 days of age, and weighing about 50 grams, divided into lots of four each and housed in a semi-darkened room. They were fed throughout the test period the Steenbock and Black No. 2965 (1925) rachitic ration. When a satisfactory rachitic condition had developed, supplements, diluted in inactive olive oil, were administered to each animal separately from a pipette.

As one of the methods uses the change of  $pH$  of the feces, considerable attention has been given to the most satisfactory manner of collection of the samples. Gathering from a pan or paper placed beneath a screen

has proved unsatisfactory, due to changes taking place in the atmosphere or by contamination with urine. As a result of many trials, it is now thought to be advisable to secure the feces direct from the animal by gently massaging the lower colon and rectum. With a little practice it is possible to collect samples from a series of animals in a few minutes. Small samples of from .3 to .8 grams have been found satisfactory, an amount that can readily be taken at any time from four animals. This makes possible the collection of the sample and the determination of the hydrogen-ion concentration within a few minutes, thus eliminating the possibilities of error due to bacterial action.

### *pH* TESTS

For the determination of the hydrogen-ion concentration Biilmann's (1921) quinhydrone electrode was employed, using as a reference half-cell .01 N HCl and .09N. KCl introduced by Viebel (1923). This makes a convenient and constant reference electrode. Bright platinum electrodes 6 mm. square, fused in glass tubes, were used throughout the experiments. For electrode vessels 100 cc. graduated cylinders were cut at the 25 cc. mark and fitted with glass stirring loops. This permitted the use of small samples. A standard Leeds and Northrup hydrogen ion potentiometer was employed.

The most satisfactory procedure for determining the *pH* of feces received consideration. It was found that dilution and time were factors in the results obtained. From a consideration of data accumulated it is now our custom to use .3 to .8 grams fresh feces macerated in 50 times its weight of distilled water, to which is added 0.2 grams of quinhydrone. The whole is thoroughly stirred for two minutes and the reading taken. This dilution and time are recommended from a consideration of the following facts.

From an inspection of Figure 1, which represents the *pH* dilution diagram, it will be observed that very little change takes place in the *pH* from a dilution of 1 to 30 (1 part feces to 30 parts water) to 1 to 75, therefore a dilution of 1 to 50 would be satisfactory for this work, since no change takes place over a considerable portion of the curve on either side of this point. At these large dilutions a slight error, due either to variation of moisture content of sample, or to an inaccuracy in measurement of the water, would have no appreciable effect on the *pH* of the sample.

A careful study was made in connection with the drift of potential with respect to time. These data are diagrammatically illustrated in

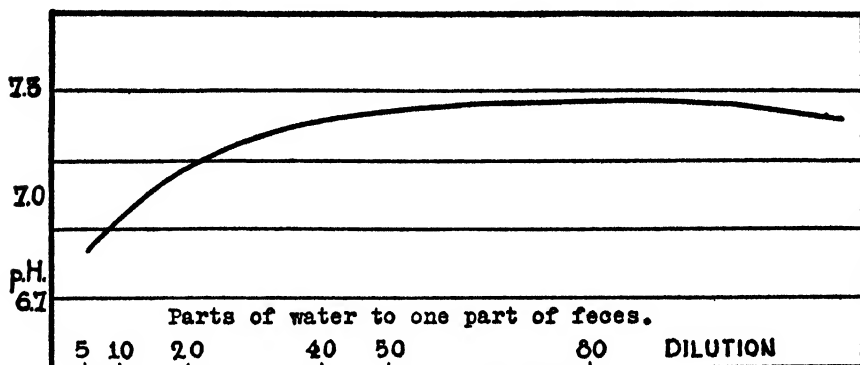


FIG. 1.—Dilution curve showing changes in  $pH$  due to varying concentrations of the fecal suspension.

Fig. 2. It will be noted that above the  $pH$  7.3 there was a tendency for the  $pH$  to fall, while at low values the tendency was to rise. If, however, the readings are made between 2 and 5 minutes after the addition of the quinhydrone, the error due to the slight drift will be negligible.

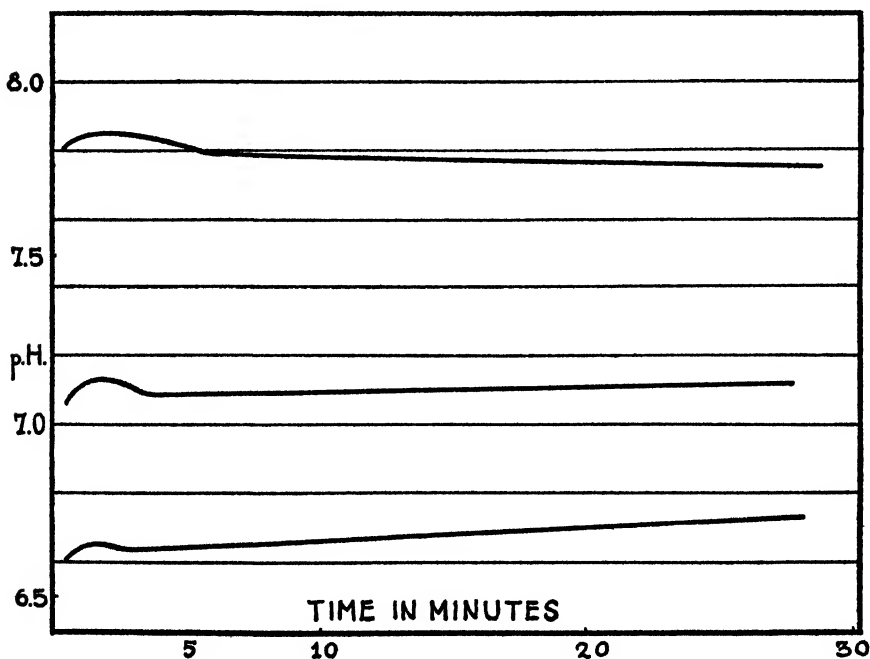


FIG. 2.—Curves showing the drift of  $pH$  as affected by time at different hydrogen-ion concentrations.

## SKIAGRAM STUDIES

In the application of Poulsson's methods certain modifications have been adopted permitting greater speed of exposures and decreasing the cost. A Fisher X-ray outfit using 10 milliamperes through a four inch gap was employed. Exposures of the left hind knee joint were made at 25 inches for a period of 5 seconds. One operator holds the rat in his left hand, the foot in his right. The animal is turned on its back and firmly held so that the left hind leg is stretched over an ordinary dental X-ray plate. The second person operates the X-ray machine. The plates are numbered to agree with the animal by the use of perforated lead plates. By this procedure an exposure each minute is easily possible.

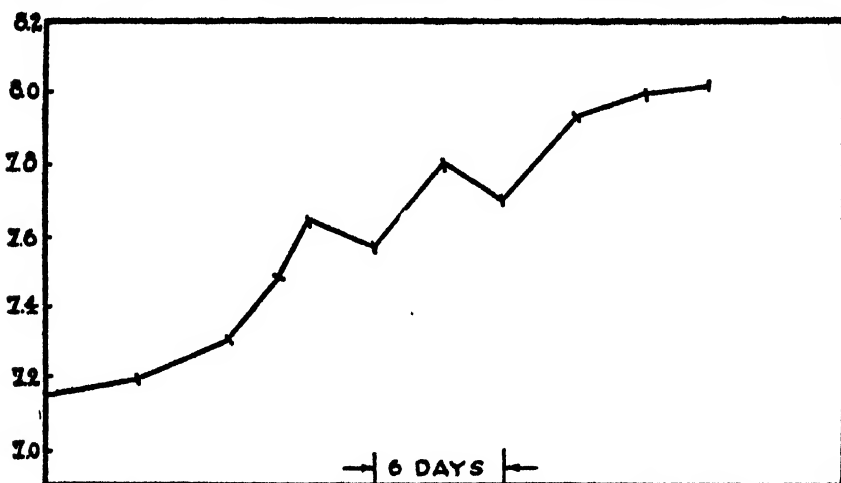


FIG. 3.—Curve showing the increase in pH of feces during the depletion period.

The most satisfactory use of the hydrogen-ion method was found in predicting the most favorable time to take the pictures when it was desired to determine the relative amounts of vitamin D of different supplements.

Skiagrams secured by the above technique were found exceptionally satisfactory. The decalcified area which marked the proper time for the addition of the supplement to the basic ration was easily recognized, as noted by Poulsson. The recalcification of the metaphysis marking the complete healing was even more easily recognized.

Figure 3 illustrates the typical rise of the pH of a composite sample of feces during the depletion period. There is represented in Figure 4 the response of a number of cages of animals to various treatments following the above mentioned period. Group 1 were controls and the

curve is merely an extension of that of Figure 3. Groups 2 and 3 received cod liver oil, the former an oil of very low potency, the latter a fresh, high grade, oil. Group 4 received an active oil concentrate. The fall of the curves gives evidence for these conclusions.

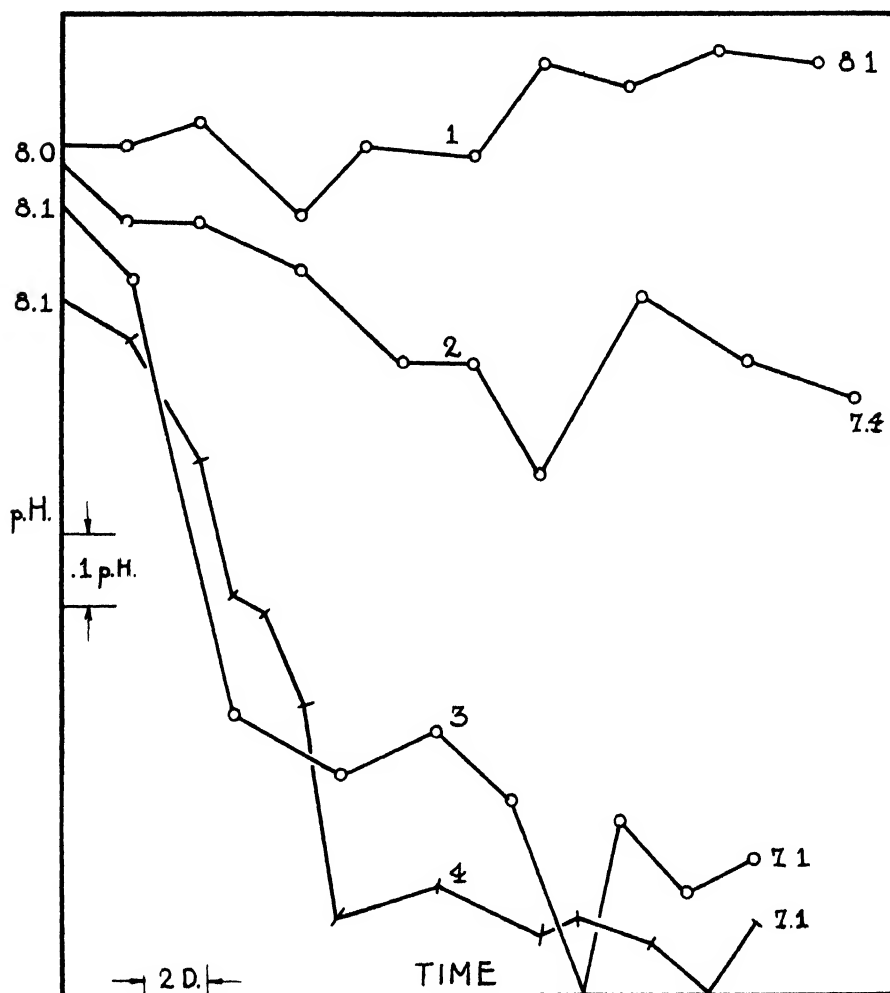


FIG. 4. Curve showing the pH of feces of four groups of animals. No. 1 was a control lot. Nos. 2, 3, and 4 received supplements of varying potencies.

Figure 5 shows the skiagrams of the same lots of animals. The upper row of pictures portray the rachitic condition at the end of the depletion period and previous to the addition of the supplements. The lower row of pictures represents the condition of the same animals at the end of test period. Nos. 1, 2, 3 and 4 are skiagrams of the same animals



described as groups 1, 2, 3 and 4 under the previous discussion of  $pH$  of the feces.

The results are typical of over 60 cages of animals checked in parallel by the two methods.

It is interesting to note that calcification does not take place at the end of the time when the  $pH$  curves begin to flatten for the first time,

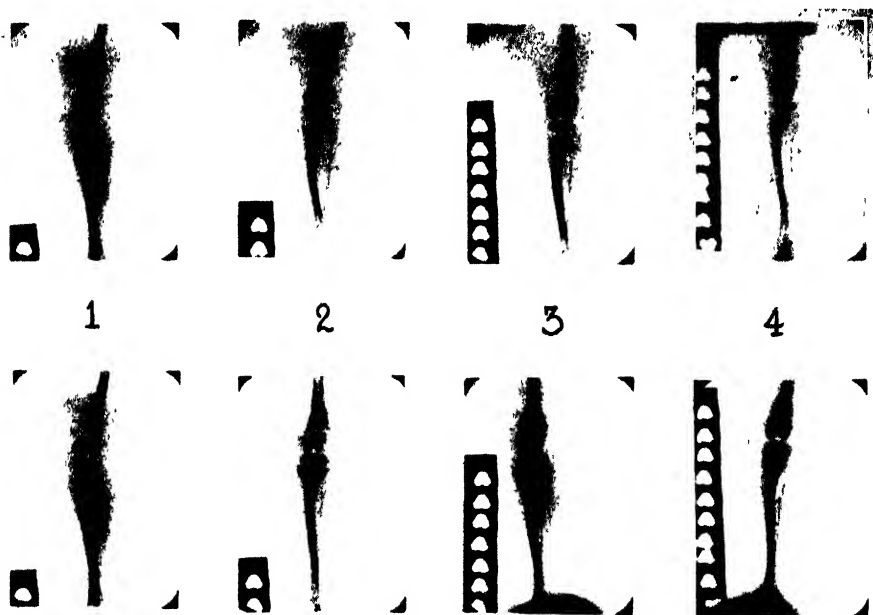


FIG. 5 The upper row of skiagrams was taken at the end of the depletion period. The lower at the end of the test period. No. 1 is of controls, 2, 3 and 4 received supplements of varying potencies

but follow it about 2 or 3 days, the change in  $pH$  being associated with the conditions that produce healing rather than the conditions resulting from the healing.

### CONCLUSIONS

The  $pH$  of the feces increases as the animal bodies become depleted of the vitamin and again drops after vitamin D supplements are added, as indicated by Jephcott. Unfortunately, the daily fluctuations are erratic and smooth curves are never obtained. During the course of this work two groups of investigators, Shohl and Bing (1928) and Oser (1928), have reported this fact and have drawn the conclusion that the

method, therefore, has no practical application. In this conclusion we concur in that we do not feel justified in arriving at complete conclusions as to the relative potencies of two slightly varying supplements. We have found a use for the method, however, that has saved expense and time. In our sixty tests it has been found that if a curve be drawn through the mean of the points, the relative slope of the curve is usually comparable to the findings of the skiagrams. It must be emphasized, however, that this holds true only when the same basic ration is used and that the supplements must be of similar nature, such as cod liver oil or its concentrates, and the method does not hold if supplements are added that change the basic ration materially.

The method finds its usefulness in combination with the Poulsson method. When the  $pH$  curve ceases to rise and flattens out, one recognizes the proper time for the first X-ray exposures, and again, after the addition of the supplements the drop of the curve and finally the reflattening, indicate the time for the second exposures. Our practice is to wait two days after the break of the curve of the animal receiving the most potent supplement, and then take the pictures of the entire series to obtain the most comparable results. An examination of the curves indicates that the drop of the  $pH$  does not parallel the recalcification, but precedes it by three or four days. At least, during the period of greatest drop of  $pH$ , no evident changes are shown in the skiagrams. The combination of the two methods makes it possible to keep a daily record of changes of a large series of animals throughout the entire period with a comparatively small amount of labor and expense, and the results are more satisfactory than the conclusion based only upon a single examination at the end of the period.

#### BIBLIOGRAPHY

- Billmann, E., and Lund, H., *Ann. Chim. phys.*, 1921, (9) XI, 109.  
Jepchott, H., and Bacharach, A. L., *Biochem. Jour.*, 1928, XXII, 60.  
Oser, B. L., *Jour. Biol. Chem.*, 1928, LXXX, 487.  
Poulsson, E., *Biochem. Jour.*, 1928, XXII, 135.  
Shohl, A. T., and Bing, F. C., *Jour. Biol. Chem.*, 1928, LXXIX, 269.  
Steenbock, H., and Black, A., *Jour. Biol. Chem.*, 1925, LXIV, 263.  
Viebel, S., *Jour. Chem. Soc.*, 1923, CXXIII, 2203.





## THE INFLUENCE OF A LOW-FAT DIET UPON FAT METABOLISM DURING LACTATION

BY L. A. MAYNARD AND C. M. McCAY

*(From the Laboratory of Animal Nutrition, Cornell University, Ithaca)*

Received for Publication—June 10, 1929

WHILE there are many experimental data indicating that milk fat has its origin in part at least in dietary fat, it is also recognized as a result of the experiments by Jordan and coworkers (1, 2) that with a low-fat diet carbohydrate can be utilized as a source of the fat in the milk. These experiments raised the question, which has been little studied and never satisfactorily answered, as to whether the substitution of carbohydrate for fat as a source of milk fat has any influence on the quantity or quality of the secreted product. The present investigation was undertaken to study this question and to obtain data which might result in a better understanding of the physiology of fat metabolism during lactation.

There are recorded in the literature a large number of experiments dealing with the influence of food fat upon the secretion of milk fat. Insofar as the influence on the quantity of secretion is concerned, the results as a whole are contradictory and inconclusive. A review of the earlier work is given by Basch (3) and by Porcher (4). Many of the experimental data reported with low-fat diets are inconclusive either because of the short duration of the experiment, or because of the presence of other variables such as differences in protein or energy intakes in the rations compared.

In the studies with cows by Jordan and coworkers (1, 2) in which the ration was made low in fat by benzine extraction, it was demonstrated, by determining the amount of fat digested and the nitrogen balance, and by other data, that the fat secreted could not have come from fat in the food or depots or from protein, but must have been made in part at least from carbohydrate. These experiments have been cited by some reviewers as evidence that the fat content of the diet is of no importance for lactation. Such a conclusion is not justified by the data. In fact, in the first report the data show that the substitution of the extracted feed resulted in a lower yield of milk and fat. However, this result is inconclusive because in changing from the unextracted to the extracted ration the intake of digestible nutrients was lowered. The writers themselves did not draw any conclusions as to whether or not a low-fat diet is unfavorable to milk and fat secretion. The importance of their work lies in the clear demonstration that milk fat can be made from carbohydrate.

Morgen, Beger and Fingerling (5, 6, 7) have reported a very large number of studies with sheep and goats from which they conclude that while carbohydrate can be utilized to manufacture milk fat, fat itself is a more suitable material and that within certain limits the percentage of fat in the milk is influenced by its content in the feed. Specifically, it was found that rations containing approximately 1 gram of fat per kilo live weight resulted in a larger secretion of fat and, to a lesser degree, of milk, than rations containing 0.5 of a gram of fat per kilo live weight. Though fairly consistent the differences obtained were small in most cases. The significance of many of the data is questionable because the rations compared differed as regards their ingredients and also as regards the quality of their protein and other factors. Since the secretion of milk and fat by different animals, which were similar in weight, varied as much as 500 per cent, the use of live weight as a basis of fat intake also tends to obscure the significance of the results.

In some additional studies by Fingerling (8) with goats, carried out similarly to those just described, the same conclusions are drawn. In these experiments the intakes of digestible protein and of starch value were kept constant, but again one feed was substituted for another in shifting the fat intake, and live weight was used as the basis for the latter.

The data reported in the papers just reviewed resulted in the inauguration of a cooperative investigation of the question with cows, carried out in ten agricultural institutions in Germany. This investigation has been reported by Kellner and coworkers (9). The rations compared were equal in "starch value," but one was richer in carbohydrate and the other richer in fat. The former contained approximately 0.5 of kilo of digestible fat per 1000 kilos live weight and the latter approximately 1 kilo per 1000 kilos live weight. The higher fat level was provided by substituting rice meal for barley meal and starch, which were used as concentrates in the low-fat ration. The data obtained failed to show any higher yield of milk or fat as a result of the high-fat ration. An analysis of the rather variable data shows that even the low-fat rations contained nearly as much fat as was secreted in the milk. In fact some of them contained more. Thus, the experiments can not be considered a rigid test of the effect of a low-fat ration.

#### EXPERIMENTAL PROCEDURE

Four cows were alternately fed a typical dairy ration consisting of alfalfa hay, beet pulp and a grain mixture, and the same ration from which

most of the fat had been removed by extracting the grain mixture with benzine, the extracted fat being replaced by an isodynamically equivalent amount of starch. During a period of 30 days two cows were fed the normal-fat ration and two were fed the low-fat ration. The rations were then interchanged for another period of 30 days, and then shifted back to the original basis for a final period of 24 days. This system of feeding is shown in the charts in which the results are presented.

The rations used were chosen with the object of providing optimum nutrition, aside from the possible effect of the removal of the fat. The hay used was second-cutting alfalfa of excellent quality. The beet pulp was used as a succulent feed by soaking it for several hours prior to feeding. The grain mixture was made up according to the following formula:

150 pounds cottonseed meal, 43% protein  
 150 pounds linseed meal  
 450 pounds hominy feed  
 250 pounds wheat bran

A sufficient amount of the above was mixed prior to the start of the experiment and divided into two portions. One portion was set aside as the normal grain mixture. The other was extracted by the benzine process,<sup>1</sup> and the fat removed, as shown by analysis before and after extraction, was replaced by an isodynamically equivalent amount of starch.

The analyses of the various feeds are shown in Table I. The figures for digestible nutrients are calculated values based on the average coefficients of digestibility published by Henry and Morrison (10).

TABLE I  
ANALYSES OF FEEDS

Feed	Crude protein %	Carbohydrates		Fat %	Dig. crude protein %	T.D.N. %
		Fiber %	N.F.E. %			
Normal grain mixture.	19.53	7.34	53.96	5.78	14.70	73.06
Low-fat grain mixture.	19.20	7.07	60.08	0.66	14.42	68.65
Beet pulp .....	9.24	20.32	59.12	0.64	4.80	70.77
Alfalfa hay .....	15.52	28.37	37.10	2.25	11.81	53.77

The cows used were mature Holsteins, at least 2 months along in their lactation period at the beginning of the experiment. All were bred shortly

<sup>1</sup> The extraction was carried out by Oil Processes, Inc., Harrison, New Jersey.

before the start of the experiment with the exception of one, number 4, which was bred 10 days thereafter. Their production for 30 days before going on experiment is shown in Chart 1. Their ration during this period had consisted of mixed hay of rather poor quality, corn silage and a 20-per-cent-protein grain mixture. The ration for each cow was adequate for her weight and production, according to the Morrison standard.

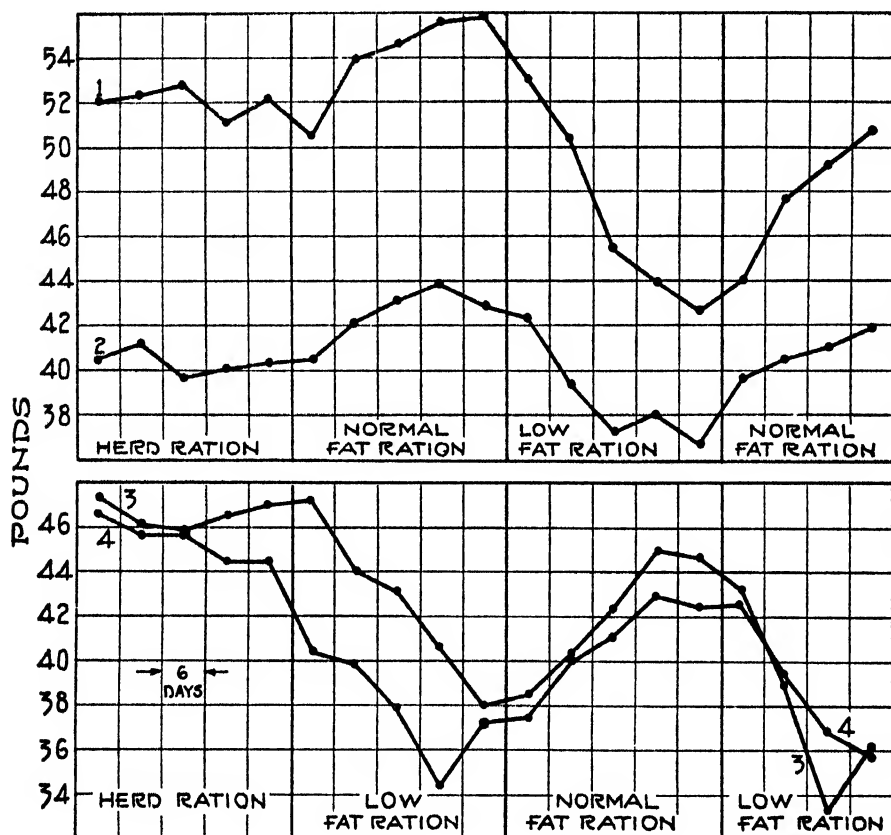


CHART 1. Average daily milk yield.

During the experiment the ration fed each cow was recalculated every 6 days. It was based upon her production of milk and fat during the previous 6 days and upon her average body weight for 3 consecutive days during this period. The normal-fat grain was fed at the rate of 1 pound for every 3 pounds of milk produced. Since the low-fat grain contained approximately 6 per cent less total digestible nutrients as shown in Table I, due to the substitution of starch for fat, it was fed at the rate of 1.06

pounds for every 3 pounds of milk. Each cow received approximately 1 pound of hay and 0.6 pounds of beet pulp per 100 pounds liveweight, but the ratios were varied as necessary to provide each cow with total digestible nutrients in accordance with the Morrison standard and with digestible protein approximately 20 per cent in excess of this standard. In deciding to use alfalfa hay with the 20-per-cent grain mixture it was, of course, recognized that the plane of protein intake would be unnecessarily high. There seemed to be no disadvantage in this and the use of the best roughage appeared distinctly advantageous from the standpoint of providing a ration optimum in all respects other than its fat content.

The cows were milked at 5 A.M., 1 P.M., and 7 P.M. The grain and beet pulp were fed before each milking and the hay was fed at 9 A.M. and 4 P.M. The beet pulp was soaked several hours and the grain was mixed with it just before feeding. Approximately 1.5 ounces of salt were fed to each cow daily. All feed was accurately weighed and any refused feed was accounted for. With the exception of one short period in the case of two of the cows, as will be mentioned in detail later, the rations were completely consumed.

The milk was sampled at each milking, taking 1 cc. for each pound produced. These subsamples were composited over a period of 6 days, analyzed for fat by the Roese-Gottlieb method and the iodine number of the fat determined by the Hanus method.

Blood samples were taken approximately every 10 days throughout the experiment. The samples were taken from the jugular vein, always at the same hour, 11 A.M. The blood was analyzed for fatty acids by the oxidation method of Bloor (11) and for cholesterol by Bloor's colorimetric method (12).

The cows were weighed on 3 consecutive days during each period of 6 days and the average taken as the weight for the period.

## RESULTS

The daily milk yields were averaged for periods of 6 days and these average values are plotted in Chart 1. To show the previous production of the animals, their yields are plotted for 5 six-day periods prior to the start of the experiment. In studying this chart and succeeding ones it should be noted that each point is plotted in the middle of the six-day period for which it represents an average value. The change to the experimental rations was made abruptly. It is noted in the chart that the yields of cows 1 and 2 rose when the animals were changed to the normal-fat ration, and that when they were changed to the low-fat ration their



yields dropped sharply and continued to drop throughout this period. When the cows were put back on the normal fat ration, it is seen that their yields started up again and continued to rise until the end of the experiment. The curves for cows, 3 and 4 show that these animals responded to the changes in the fat content of the ration in an entirely similar way. When changed from the herd ration to the low-fat ration their yields dropped sharply, rose nearly to their original levels on the normal and dropped again sharply during the final low-fat period. The fourth value plotted for cow 4 during the first low-fat period is abnormally low because during a portion of the 6 days represented by this value the animal was suffering from a sore on her jaw and thus failed to eat all her food. The disappearance of the trouble is reflected in the higher yield shown for the final 6 days of the period. Similarly the sharp dip and subsequent rise in the curve for cow 3, at the end of the final low-fat period, was caused by the animal's going off-feed. During the third period of 6 days this animal consumed only about half her ration and thus her yield fell off markedly. In the succeeding period she ate her entire ration and thus her yield came back. Aside from these two irregularities in the curves for cows 3 and 4, which are entirely explainable on the above basis, the curves are remarkably uniform in showing that the change in the fat content of the ration had a marked effect upon the yield of milk.

To make more certain regarding this conclusion, it is desirable to know whether the intakes of total digestible nutrients during the different periods were adequate and similar in accordance with production. The data in Table II furnish an answer to this question. The figures for the total digestible nutrients required by the Morrison standard were computed on the basis of the cow's average weight and her yield of milk and fat during the period represented by each ration. The figures for the actual intake were obtained from the records of food consumption and the data as to percentage composition of each feed, using the average digestion coefficients as published by Henry and Morrison (10) to compute the total digestible nutrients. These data for the theoretical requirements and for the actual intakes could have been computed for each six-day period, but this did not seem necessary for the purpose. For the data as a whole the correspondence between the required intake and the actual intake is remarkably close.

Assuming the substantial accuracy of the feeding standard and the applicability of the average digestion coefficients, it is clear that the drops in production which occurred on the low-fat ration and the increases that occurred on the normal were not due to changes in total food intake and

they must have been due to the changes in the fat intake. The only low-fat period during which the actual intake was significantly less than the theoretical requirement, was the last one for cow 3. This was due to her failure to consume the ration allotted her during one period of 6 days, as previously explained.

TABLE II  
DAILY INTAKES OF TOTAL DIGESTIBLE NUTRIENTS COMPARED TO REQUIREMENT  
BY MORRISON STANDARD, AND DAILY INTAKES OF DIGESTIBLE FAT.

Cow	Ration	Total Digestible Nutrients		Digestible Fat lbs.  lbs.
		Requirement by Morrison Standard lbs.	Actual intake  lbs.	
1	normal	23.6	23.4	1.02
	low-fat	22.4	22.3	0.23
	normal	23.2	23.3	0.94
2	normal	23.3	23.2	0.87
	low-fat	22.4	22.6	0.24
	normal	23.7	24.0	0.85
3	low-fat	23.6	23.9	0.25
	normal	24.9	24.0	0.87
	low-fat	23.2	22.5	0.24
4	low-fat	20.0	20.0	0.21
	normal	21.0	20.9	0.81
	low-fat	20.4	20.6	0.22

In drawing the conclusion that the production dropped as a result of the lower fat intake and despite an adequate intake of total food, the possibility is recognized that it may have been due to the removal of some other dietary essential besides fat. This possibility seems remote. While some fat-soluble vitamins were doubtless removed, previous studies indicate that this would affect the content of the milk in these vitamins rather than the yield. It is also possible that the removal of the fat lowered the digestibility of the entire ration and that the intake of total digestible nutrients was actually inadequate, although shown to be adequate on the basis of the calculation using average coefficients. While this may deserve study by including digestibility determinations in a repetition of the experiment, the possibility that the digestibility was sufficiently lowered to cause the large drop in yield recorded seems remote.

Table II also contains the figures for the average daily intakes of digestible fat for the different periods. Average digestion coefficients were used in computing these values. It is seen that the intake of digestible fat was reduced by approximately 70 to 75 per cent when the low-fat ration was substituted for the normal.

The curves in Chart 2 show that the change from one ration to another was without effect on the weights of the animals.

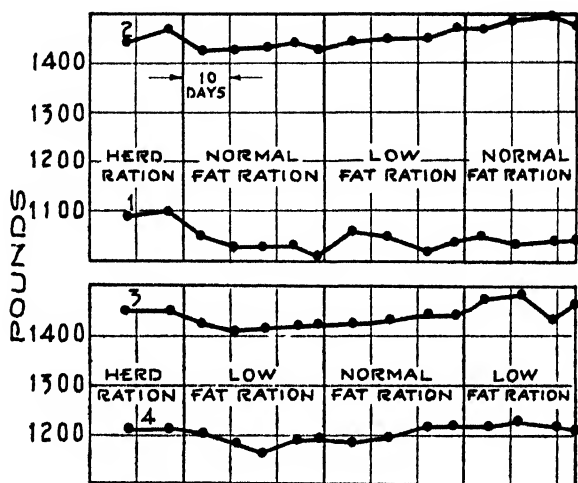


CHART 2. Weights of cows.

The data obtained by analyzing the six-day composite samples of milk for fat are shown in Chart 3. The data show a high degree of variability. It has long been recognized that fat is the most variable constituent of milk. Clearly there is no indication in Chart 3 to support the conclusion of Morgen and coworkers (5, 6, 7) and of Fingerling (8) that a low-fat ration lowers the percentage of milk fat. On the contrary, if the curves show anything at all, it is a tendency for the percentage to rise on the low-fat ration and to fall on the normal. However, any such tendency should not be considered a direct effect of the changes in the fat intake. Rather it may be considered a result of the changes in yield. There are many data showing that as the yield of milk falls, the percentage of fat tends to rise. Particularly this is noted toward the end of the lactation and at other times when the yield suddenly drops for any reason. The high value obtained for cow 3 in the next to the last six-day period is an illustration of this since, as is shown in Chart 1, this is the period during which her milk yield took a very sudden drop as a result of failure to eat.

Many of the conflicting data in the literature are due to the failure to recognize the normal variability in the fat content of milk and the frequent inverse relationship of this percentage to the yield.

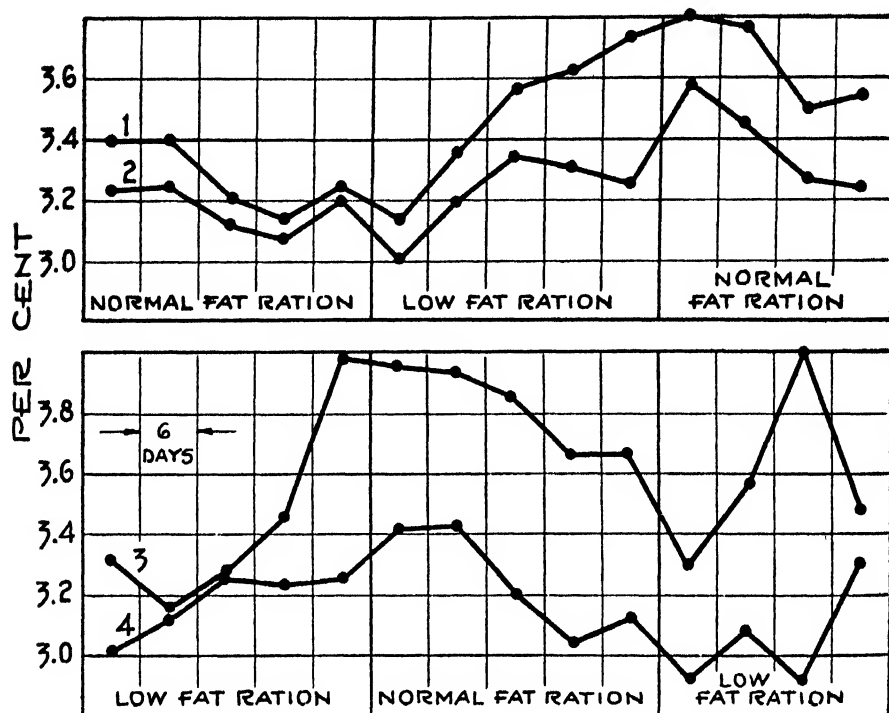


CHART 3. Percentage of fat in milk.

The curves for the daily yield of fat are shown in Chart 4. Although these curves are irregular, as is to be expected in view of the variability of the percentage of fat, it is apparent that the yield of fat tended to be lower on the low-fat ration. It is clear from the data presented in charts 1 and 3, however, that this lower fat yield is due to a lower yield of milk as a whole, and not to a lower percentage of fat. While at first thought this may seem difficult to understand, it should be remembered that, though the fat percentage may vary within certain limits under the influence of a variety of factors, the mammary gland tends to secrete a product of constant composition. On this basis a lack of suitable raw material for the manufacture of a given nutrient will tend to reduce the secretion as a whole.

A comparison of the data in Table 2 for the intake of digestible fat with the data presented in Chart 4 shows that the normal fat ration contained

approximately 60 per cent as much digestible fat as was secreted in the milk, whereas the low-fat ration contained only about 18 per cent as much. It is a question worthy of study whether or not the milk secretion would be increased beyond that obtained on the normal ration by increasing its fat content so that the animal would receive as much fat in her feed as is required for the milk secreted.

The curves for the lipids in the blood plasma are shown in Chart 5. The curves for both the fatty acids and cholesterol show that when cows 1 and 2 were changed to the experimental ration of normal-fat content the lipids in the blood tended to remain unchanged, that they dropped

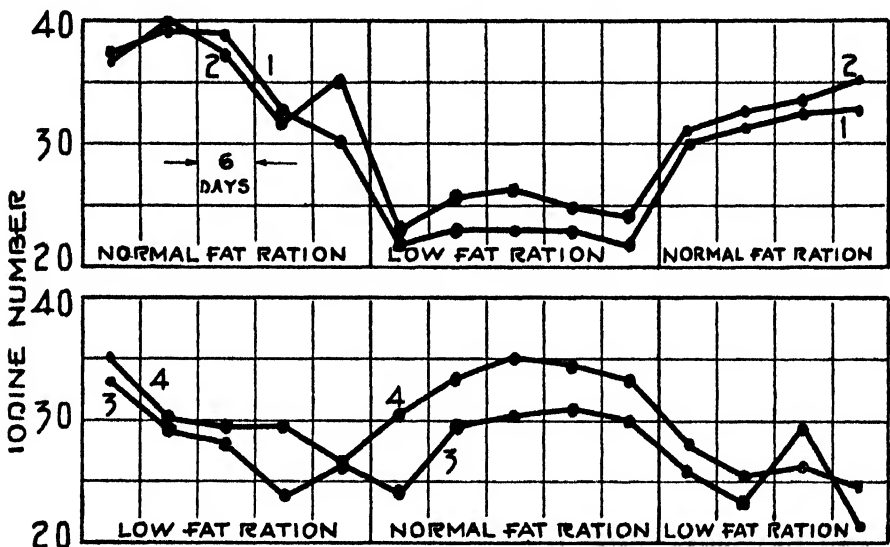


CHART 4. Average daily yield of fat.

sharply when the low-fat ration was substituted, and continued to drop until the normal ration was again fed. They then gradually rose to their previous normal values. The values for cows 3 and 4 are seen to drop during the low-fat period, rise during the normal, drop again during the second low-fat period, and return to normal when placed again on the herd ration. The latter contained somewhat more fat than the normal-fat experimental ration.

It is noted that there is a close parallelism between the curves for fatty acids and cholesterol similar to that shown by Terroine (13) for dogs. There is also a striking parallelism between the trends of the curves in Chart 5 and those for milk yield shown in Chart 1. It should be noted that the drop in blood lipids on the low-fat ration and the subsequent rise when the

ration higher in fat was substituted is not due solely to alimentary fat. The full influence of the latter should be shown at the first observation in the period in question, since this observation was not made until 7 or 8 days after the change, whereas it is seen that the drop or rise continued

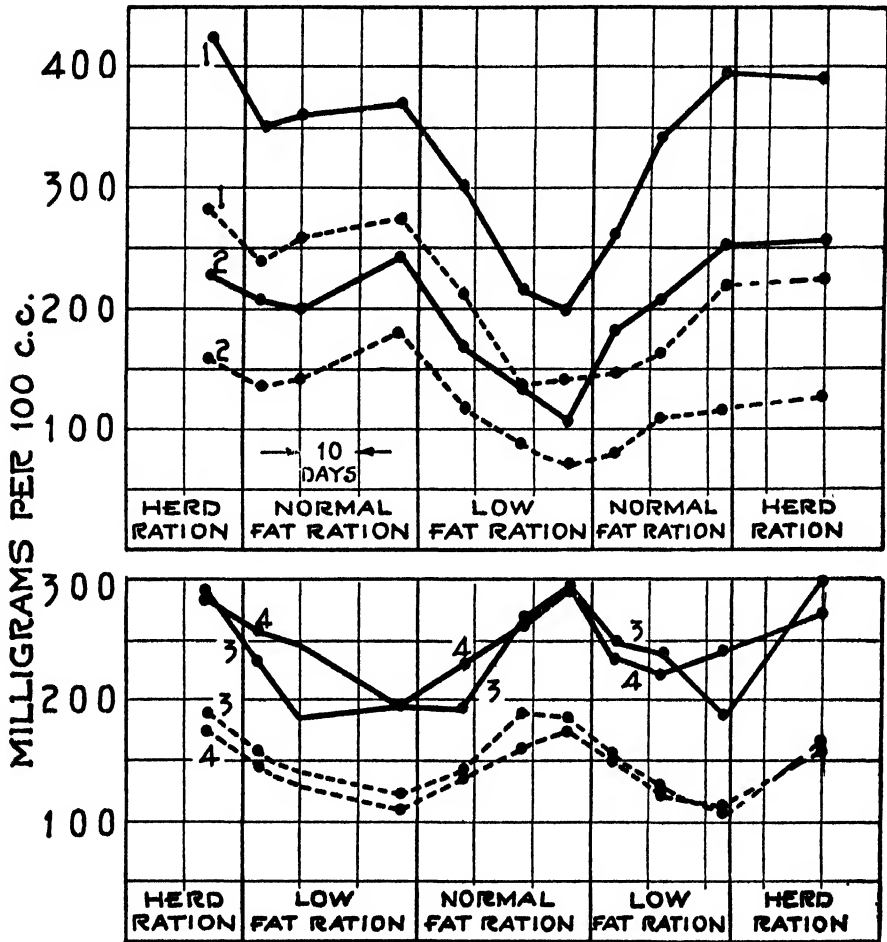


CHART 5. Fatty acids and cholesterol in blood plasma—— Fatty Acids- - -Cholesterol.

throughout the period. The significance of these changes in blood lipids will be further discussed later.

In view of the fact that in the dog and other animals studied there is a marked rise in blood lipids following the ingestion of food containing fat, and the fact that constant values as well as a parallelism between fatty acids and cholesterol are found only in the post-absorptive state, the question naturally arises as to why the curves in Chart 5 are so uniform.

If the level of blood lipids in the cow is as greatly affected by food consumption as has been shown for the dog, then the results in Chart 5 would seem rather fortuitous and the significance that has been attached to them in the previous discussion would seem questionable. Since the normal ration of the cow contains only a small percentage of fat compared to that of the dog, and since digestion and absorption in the ruminant is a fairly continuous process, particularly where food is given 5 times a day as was the case in the present experiment, it seems, on theoretical grounds, that the level of the blood lipids would be comparatively little affected by the feeding of a ration uniform in amount and composition. The writers have experimental evidence to substantiate this view.

The senior writer, working with Professor Porcher, studied the lipids in the blood of milking cows by taking samples at various hours after feeding and also after milking. The maximum variation found was approximately 20 per cent, the average variation was much less, and such variations as occurred could not be correlated with either feeding or milking (14). In Table III there are presented some similar data obtained with cows 1 and 2

TABLE III  
LIPIDS IN BLOOD PLASMA BEFORE AND AFTER FEEDING. MILLIGRAMS PER 100 cc.

Hour	Cow 1		Cow 2	
	Fatty Acids	Cholesterol	Fatty Acids	Cholesterol
5 A.M.....	398	200	226	122
7 A.M.....	380	206	223	124
11 A.M.....	395	220	253	116

during the present experiment. At the time, these cows were receiving the normal ration. The first samples were taken at 5 A.M., 10 hours after the last feeding. The cows were then fed their grain mixture and two other blood samples were taken 2 and 6 hours later. The data show no rise in blood lipids following the intake of food and a maximum variation of around 15 per cent.

The results of the determinations of the iodine number of the milk fat are shown in Chart 6. The values represent determinations on the six-day composite samples of milk. The data show clearly that the iodine numbers were lower with the low-fat ration, indicating a distinct change in the character of the fat secreted. It is well understood from the work of several investigators reviewed by Terroine (15, p. 96) that the character

of the milk fat is influenced by the character of the food fat, that as the iodine number of the food fat increases, the iodine number of the secreted fat tends to rise. Engel (16) and Henriques and Hensen (17) have shown that inanition results in a rise in the iodine number of milk fat and Eckles and Palmer (18) have reported similar results for underfeeding.

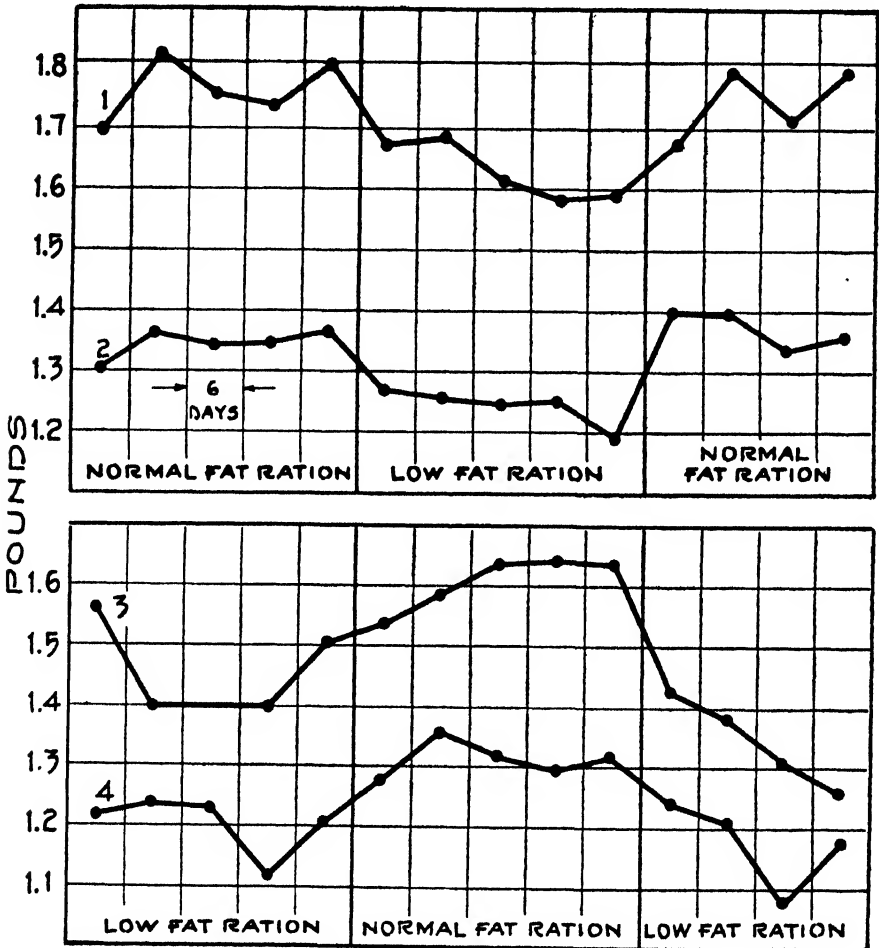


CHART 6. Iodine number of milk fat.

These findings coupled with the observation by Engel (16) and by Eckles and Palmer (18) that deposit fat is less saturated than milk fat indicate that the iodine number of milk fat rises in inanition because the deposit fat is being drawn upon for its manufacture. In the present study, however, the milk fat has a lower iodine number on the low-fat ration. It is understood from many experiments that when carbohydrate is substituted



for fat in a fattening ration, the iodine number of the deposit fat falls. It seems probable, therefore, that in the present experiment, where an adequate supply of energy was furnished, the animal when on the low-fat ration did not draw upon its reserves to any marked extent for a source of milk fat, but used instead the food carbohydrate for the purpose.

One might expect that with a lowering of the food fat there would be a tendency to mobilize deposit fat. While this may have occurred to a certain extent, it does not seem that it was a large factor or that the decline in the blood fat was primarily due to this decreasing mobilization, in view of the rapid drop in the iodine number of the milk fat when the cows were changed to the low-fat ration. The cows were in rather thin condition. Perhaps if their bodies had contained a large amount of soft fat, such as is present at the beginning of lactation in a cow previously liberally fed, the results would have been different.

The experiments of Foa (19) and Meigs (20) are in agreement that the blood precursor of milk fat is a lipid although they differ as to the lipid concerned. It is understood that in the secretory process a certain selective action and a modification of the blood fat occur in the mammary gland. It is also clear that this blood lipid may have its origin either in deposit fat or in food fat or in carbohydrate. On a ration rich in fat it seems probable that the food fat is the principal source. In the present experiment it seems likely that food carbohydrate was called upon to supply the fat not supplied by the food fat. The results suggest that the carbohydrate was not as suitable or useful a source as the food fat for meeting the requirement of the mammary gland, with the result that two things happened; the lipids in the blood were gradually reduced, and the secretion of milk dropped due to a deficient supply of one of its precursors. As has been previously mentioned, the curves showing the drop in blood lipids and in milk fat tend to parallel each other. No conclusions can be drawn from the present experiment as to the most suitable plane of fat intake for maximum milk secretion. The investigation is being continued with this question as one of the objects of study.

The same rations as used in the experiment with cows were fed to goats for periods of approximately 15 days and single determinations of the blood lipids and of the iodine number of the milk fat were made in the different periods. By themselves the data, shown in Table IV, are too meager to be of value, but it is significant that they show the same trend as the cow data and thus tend to confirm the conclusions drawn. They also indicate that goats may be suitable animals for a further study of this problem.

TABLE IV  
LIPIDS IN BLOOD PLASMA AND IODINE NUMBER OF MILK FAT—GOATS

Goat	Ration	Mg. per 100 cc.		Iodine no. of milk fat
		Fatty acids in blood	Cholesterol in blood	
1	normal	169	93	26.0
	low fat	145	56	20.5
	normal	183	75	28.0
2	normal	193	97	30.0
	low fat	171	63	25.0
	normal	232	82	28.5

### SUMMARY

Experiments to determine the influence of food lipids upon the milk secreted, have been carried out employing the cow and goat as experimental animals. A ration from which most of the fat had been extracted produced a marked lowering in the volume of milk secreted without any significant alteration in its fat content. The fat secreted in the milk upon a low-fat ration, had a lower iodine number than that produced upon a normal ration. The decreasing milk secretion on the low-fat diet was accompanied by a gradual decrease in the blood lipids.

### REFERENCES CITED

1. Jordan, W. H., and Jenter, C. G., *N. Y. Agr. Expt. Sta. Bull.*, 1897, 132.
2. Jordan, W. H., Jenter, C. G., and Fuller, F. D., *N. Y. Agr. Expt. Sta. Bull.*, 1901, 197.
3. Basch, K., *Ergeb. d. Physiol.*, 1903, II, 326.
4. Porcher, Ch., *Le Lait*, 1926, VI, 1.
5. Morgen, A., Beger, C., and Fingerling, G., *Landw. Vers. Sta.*, 1904, LXI, 1.
6. Morgen, A., Beger, C., and Fingerling, G., *Landw. Vers. Sta.*, 1905, LXII, 251.
7. Morgen, A., Beger, C., and Fingerling, G., *Landw. Vers. Sta.*, 1906, LXIV, 93.
8. Fingerling, Gustav., *Landw. Vers. Sta.*, 1906, LXIV, 299.
9. Kellner, O., Neubauer, H., Pfeiffer, Th., Schmöger, M., Wagner, P., Immendorff, H., Weigmann, H., Schneidewind, W., Loges, G., Kleemann, A., and Henkel, Th., *Untersuchungen über die Wirkung des Nahrungsfettes auf die Milch production der Kuhe*. Berlin 1907.
10. Henry, W. A., and Morrison, F. B., *Feeds and Feeding*, 18th edition, Madison, Wis., 1923.
11. Bloor, W. R., *Jour. Biol. Chem.*, 1928, LXXVII, 53.
12. Bloor, W. R., *Jour. Biol. Chem.*, 1917, XXIX, 437.
13. Terroine, Emile F., *Jour. Physiol., et Path. gen.* 1914-15, XVI, 212.
14. Porcher, Ch., and Maynard, L. A., Unpublished data from *l'Ecole Veterinaire*, Lyon, France.
15. Terroine, Emile F., *Ann. Sci. Nat. Zool.*, 10<sup>e</sup> Serie., 1919, IV, 1.
16. Engel, I., *Arch. f. Kinderheilk.*, 1906, XLIII, 194, 204.
17. Henriques, V., and Hansen, C., *44 Beretn. f. d. k. Vet. og Landbohøjsk. Laborat. f. landökön. Forsøg.*, Kjøbenhavn, 1899, 1-30.
18. Eckles, C. H., and Palmer, L. S., *Mo. Agr. Expt. Sta. Res. Bull.*, 1916, 25.
19. Foa, Carlo, *Arch. d. Fisiol.*, 1911-12, X, 402.
20. Meigs, E. B., Blatherwick, N. R., and Cary, C. A. *Jour. Biol. Chem.*, 1919, XXXVII, 1.





# THE NUTRITIVE VALUE OF CEREAL BREAKFAST FOODS

## I. COMPOSITION AND HEAT VALUE

By

JOHN R. MURLIN, WILLARD R. LINE, H. A. PIPER, AND H. B. PIERCE

*(From the Department of Vital Economics, University of Rochester,  
Rochester, N. Y.)*

Received for Publication—June 5, 1929.

THE nutritive value of cereal foods has been extensively studied in the past, but since the publication of Woods and Snyder's bulletin (1906) and Harcourt's paper (1907) there has been no general survey of the cereal breakfast foods in common use in comparison with one another and scarcely any study exists where the human subject has been used for comparison of these articles with other staple articles of diet. Meantime many new methods of study have been devised and new conceptions of food values have been acquired. The modern conception of the biological value of protein dates from 1909, and the entire, enormous literature on the vitamins has accumulated since 1912. Many conclusions regarding the vitamin values and protein values of cereal foods have been established by means of experiments on lower mammals; but there remains a considerable number of questions of nutritive value, both within and without these fields, which can be appropriately studied on the human subject. In the semi-scientific literature on foods and diets there are found not infrequently hints that the "super milling" and other processing, such as the sterilization with heat, steaming in preparation for rolling, crushing, toasting, crisping, and the like, to which our package breakfast foods are subjected, are really devitalizing in their effects and therefore destructive of important nutritive properties. Is this a correct picture, or to what extent have the nutritive properties been affected by the modern milling and packing methods?

The opportunity to make a comprehensive study of this class of foods, which apparently has become a fixed element in the dietary of the American people, came in the form of a grant of money to this laboratory by one of the large producing companies a few years ago, the only stipulation on the part of the company being that their own product should be included in all phases of the study, and the stipulation on the part of the laboratory being that the results, insofar as they might be considered of general scientific interest, should be truthfully published. Aside from

this concern with the general question of the effects on the nutritive properties of this class of foods, the laboratory has had two other interests: (1) To determine what might be gained by newer methods of study, some of them originally devised for quite different purposes, and (2) to make a comparison of results obtained on the rat with results of the same kind of study on the human subject. The first of these interests related more particularly to the application of the Folin-Wu (1919) sodium tungstate method of precipitation of the proteins as an aid in the study of both protein and carbohydrate digestion in glassware, to the retention tube method of Rehfuess (1914), and Rehfuess, Hawk & Bergeim (1914) for studying gastric digestion, to the respiratory quotient as a measure of carbohydrate digestion and absorption, and to the dynamic action of a mixed carbohydrate and protein food in comparison with a nearly pure protein.

With reference to the second interest, a feeling has prevailed in certain quarters that nutrition studies have become rather too completely identified with the rat. There are still many respects in which human nutrition might be supposed to differ from that of the rodent. At all events, we need reassurance from time to time that what has been established for the rat is really true of man, so far, at least, as might be expected. Is the biological value of protein the same for the rat as for man, and is the rate of digestion and absorption comparable in the two organisms so widely separated in the zoological scale? We have not hoped to give a final answer to these questions but at least to make a start toward certainty as to the evaluation of nutritional studies on the lower mammals.

The entire program included studies of the following phases of nutrition. I. Digestion, both *in vitro* and *in vivo*. II. Absorption: (a) Of carbohydrate as indicated by the method of the R. Q. on the human subject and by the method of Rubner (1902) as adapted by Cori (1925) to the rat; (b) Absorption of protein as indicated by the method of nitrogen elimination; (c) Total utilization, meaning difference between intake and output by the stools. III. The biological value of proteins, as compared with the proteins of milk; (a) on the human subject; (b) on the rat. IV. The vitamins and proteins necessary for growth in the rat, as supplied by typical cereal breakfast foods. V. The dynamic action of cereal foods as compared with meat. The results obtained in these various phases of the study, so far as they have yet been carried, will be published in a series of papers of which this is the first.

The study presented herewith under the subtitle of "Composition and Heat Value" represents a part of the results of two summer's work by

several assistants. The first part of the title is not very completely treated, only a sufficient number of analyses having been carried out to reassure ourselves of the nature of the particular products being studied.

All of the cereals used in these studies on composition and digestibility were package goods. Comparatively little cereal breakfast food is now obtained in bulk by the American home. The advantage of protection against weevil by sterilization and hermetic sealing, now generally practised, not to speak of the appreciation of crispness and freshness in some products, insured by the moisture-proof package, is sufficient to offset the economic gain, except in institutional nutrition, of buying by the barrel. Analyses were not available for some of these package goods.

To avoid any appearance of discrimination as between the different cereal products included in the program, it has been considered best to use descriptive rather than trade names for the several cereals. What the science of nutrition seeks to make known are, first of all, the fundamental principles of nutrition and the education of the public must take care of particular choices, in accordance with these principles.

### 1. COMPOSITION

Six different, well-known breakfast foods have been used in this series of studies. They were chosen largely because of their popularity. Two are made from oats, one from corn, and three from the wheat grain. All of these were analyzed chemically and all but one were burned in a Kroecker bomb, the heat being measured with the Riche (1913) adiabatic bomb calorimeter. The first oat product is made from the whole grain, minus the hull, by precooking and crushing between rollers. The precooking is carried out to a different extent in different products sold under the same general trade name. The one used here is steamed for a few seconds only at 60 pounds pressure to sterilize and soften the grain preliminary to rolling and will be known simply as "Rolled Oats" in contradistinction from another product steamed longer, which will be called "Precooked Oats." The corn product is made from the decorticated and degerminated corn, is treated with a small amount of cane sugar and salt and cooked under steam pressure. It is then dried, rolled, and toasted on hot rollers. It will be called "Toasted Corn Endosperm." The first wheat product mentioned in the table likewise is made from the decorticated and degerminated grain but has nothing added to it and is only sterilized by heat, not cooked, before packing. We understand from the manufacturer that it represents that part of the wheat grain which would find its way into the so-called "patent white flour," merely diverted

from the process before it reaches the final rollers. It will be called "Wheat Endosperm." The second wheat product mentioned in the paper purports to be a whole grain product, but the analyses which we have obtained do not bear out this statement on the part of the manufacturer.<sup>1</sup> Because our analyses do not indicate that this product is truly the whole grain, we have decided to call it "Toasted Whole Wheat" using the expression in quotes. The third wheat product is labeled on the package "A Whole Wheat Cereal" but again our analyses indicate that the product does not contain the entire grain, because of the low fat content. This product however is not toasted, and therefore will be described simply as "Whole Wheat" using the expression again in quotes. From microscopic examination it is evident that both of these last named wheat products do contain a liberal amount of bran and the second contains a liberal amount of the wheat germ also.<sup>2</sup>

From this statement of the mode of manufacture of each of the products under examination, we are prepared for the differences in composition presented by Table I. The two oats preparations contain the highest percentage of protein and of fat, and correspondingly less carbohydrate (starch), because they consist of the whole grain. The Toasted Corn Endosperm, because it is dried by toasting and immediately packed in wax paper, contains, as one would expect, a very small amount of moisture as it reaches the consumer. It is correspondingly high in total solids, but because the corn is degerminated, and also because sugar and salt are added, it contains very low percentages of protein and fat. The Wheat Endosperm may be described as the whole grain less the germ and bran. Nothing having been added, the percentage composition is what nature intended for the endosperm. The "Toasted Whole Wheat" has a considerably lower moisture content than the non-toasted product just mentioned, as would be expected because of the effect of heat. Curiously enough, however, it has a lower percentage of protein and of fat

<sup>1</sup> The statement of the manufacturer is as follows: "We retain as nearly as possible, with careful chemical analyses, the exact proportions of the various parts of the wheat which constitute the wheat mix on the mill at the time. In other words, we retain the endosperm, the aleurone layer, the episperm, the testa, the endocarp, the epicarp, the epidermis and the germ, or heart. The chemical analysis of the ground whole wheat product which we use is somewhat changed in our process, which is one of toasting with nothing added and nothing taken away."

<sup>2</sup> It is a curious custom amongst millers as well as amongst consumers that has sanctioned the term "whole wheat" to indicate something considerably less than the whole wheat. To be sure of getting truly whole wheat the consumer must ask for "Graham" flour. The Food Standards Committee, appointed by the U. S. Dept. of Agriculture, has proposed recently some clarifying definitions of whole wheat flour, entire wheat flour and bolted Graham flour. Similar clarifications should be applied to the cereal breakfast foods.

than the wheat endosperm alone. The "Whole Wheat" product, because of careful packing, has a low moisture content and because it contains a considerable amount of germ and bran has a high protein content, but the low fat percentage indicates that some of the germ has been removed, for again the percentage is lower than that of the Wheat Endosperm. Some allowance must be made here for different varieties of wheat and wheat grown under different conditions. The recent paper by Greaves and Hirst (1929) has sharply called attention to the wide variations in mineral content of oats, barley, corn and wheat grain grown on different soils and with and without irrigation, in the State of Utah. Similar studies are very much needed with reference to the effect of soil and particularly of fertilizers on the other nutritive properties of the cereal grains.

TABLE I  
COMPOSITION (Average Percentages)

	Moisture	Total Solids	Ash	On Dry Basis		Carboh. (by diff.)
				Protein N x 6.25	Fat (ether extr.)	
Rolled Oats	11.02	88.98	2.06	17.63	8.13	72.18
Precooked Oats	7.36	92.64	1.84	18.43	6.14	73.59
Toasted Corn Endosperm	7.49	92.51	3.07*	7.98	1.82	87.13**
Wheat Endosperm	12.68	87.32	0.74	13.52	2.75	82.99
"Toasted Whole Wheat"	7.06	92.94	1.35	12.99	1.89	83.77
"Whole Wheat"	8.08	91.92	1.45	16.49	1.47	80.59

\* Some NaCl added.

\*\* Some Sugar added.

The table presents the average percentage composition of the six cereal breakfast foods used in the following studies on the dry basis so as to make them comparable in spite of their varying moisture contents found in the package.

## 2. HEAT VALUE

How a food ranks as a source of energy can be determined approximately by burning it in pure oxygen and measuring the heat given off. With all protein foods an allowance must be made for that part of the protein molecule which is split off as ammonia and becomes converted to urea before its elimination from the body; in other words, for the fraction not oxidized. This correction alone, however, does not give the true physiological heat value, for a considerable fraction of the organic constituents may not be absorbed. This is true of the protein of cereals,



particularly if they are consumed as whole cereal. The only practicable way to obtain the heat value to the body of any article of food is to determine the percentage utilization (intake minus excretion by the feces) for each of the organic foodstuffs and then to calculate its utilization or metabolizable energy from the percentage composition, using the established heat values for the kind of protein, fat and carbohydrate ingested in that article. Atwater and his associates (1901, 1903) elaborated a method for this calculation, which is not sufficiently known, and may with propriety therefore be described here very briefly.<sup>3</sup>

The percentage utilization is obtained from analysis of the feces for protein, fat and carbohydrate, the difference between these figures and those representing the composition of the food itself (both on dry basis) being expressed as percentages of the original components. Knowing these utilization coefficients, so-called, a utilization or metabolizable heat value per gram of protein, fat and carbohydrate is obtained by multiplying the average calorimetric heat value of the kind of protein, fat and carbohydrate ingested by the utilization coefficients, and in the case of protein making a further correction for the heat value of a gram of nitrogen in the urine.<sup>4</sup> For example, the average bomb value of one gram of vegetable protein as found by the Atwater school is 5.8 Cal., for vegetable fat 9.4, and for carbohydrate 4.2 Cal. In the case of Rolled Oats, as determined in the present studies (see paper IV of this series) the utilization coefficient is 84 per cent (Table II) for protein, 90 per cent<sup>5</sup> for fat, and 96.6 per cent for carbohydrate. The utilization heat values therefore are 3.82 Cal. for the protein, 8.46 Cal. for the fat and 4.06 Cal. for the carbohydrate in Rolled Oats (Table II). The amount of heat obtained by the body from one gram of Rolled Oats (dry) is then obtained by multiplying these heat values by the amount of protein, fat and carbohydrate in a gram of the cereal (See Table I) and adding them together.

Comparison of the physiological heat values for the different cereal products with their calorimetric or combustion heat values is instructive. The latter were obtained by burning the cereals, after drying to constant weight or as obtained from the package. The directly determined values are given in the table in italics; the calculated values on dry or moist basis, from knowledge of the moisture content, are given in ordinary

<sup>3</sup> For a fuller statement the reader is referred to the senior author's review of the subject in *Endocrinology and Metabolism*, New York, 1922, Vol. III, p. 551.

<sup>4</sup> The factor for the heat value of a gram of nitrogen in the urine is 7.9 Cal. or for a gram of protein represented by this nitrogen ( $7.9 \div 6.25$ ) is 1.25 Cal.

<sup>5</sup> Not determined in these studies but adopted from Atwater (*loc. cit.*) for cereal foods generally.

TABLE II  
HEAT VALUE OF CEREAL FOODS BY BOMB CALORIMETER COMPARED WITH  
PHYSIOLOGICAL HEAT VALUE

Cereal Food	Heat of Combustion		% Moisture	Physiological Heat Value Based on Percentage Utilization* & Composition (dry basis)**				
				Coefficient of Utilization	Calories per Gram		Loss	
	Dry Basis Cal. per gm.	Moist Basis Cal. per gm.			Component	Cereal Food	Cal. per gm.	Per cent
Rolled Oats	4.761	4.320	9.27	P. 84.	3.82	0.673		
	4.807	4.361	9.27	F. 90	8.46	0.688		
	4.794	4.371	9.68	C.H. 96.6	4.06	2.928		
	Av. 4.787	4.351			Total	4.289	0.498	10.4
Toasted Corn Endosperm	4.155	3.857	7.16	P. 84	3.82	0.305		
	4.159	3.861	7.16	F. 90	8.46	0.154		
	4.218	3.894	8.34	C.H. 97.1	4.08	3.555		
	4.186	3.864	8.34					
	Av. 4.179	3.869			Total	4.014	0.165	4.1†
Wheat Endosperm	4.432	3.966	10.50	P. 94	4.28	0.578		
	4.394	3.833	10.50	F. 90	8.46	0.233		
	4.430	3.970	11.63	C.H. 96.3	4.04	3.353		
	Av. 4.414	3.923			Total	4.164	0.250	5.6
"Whole Wheat"				P. 87.2	3.97	0.655		
	4.440	4.084	8.08	F. 90	8.46	0.124		
	4.423	4.065	8.08	C.H. 96.	4.02	3.240		
	Av. 4.431	4.074			Total	4.019	0.412	9.3

\* As determined in these studies (see paper IV).

\*\* See Table I.

† This low value is due in part to the added sugar.

type. Since the physiological value was calculated on the dry basis the comparison with crude or bomb heat value can be made only on this basis.

For the Rolled Oats the loss in heat value from non absorption and non combustion (of protein) is 0.498 Cal. per gram or 10.4 per cent. For the Toasted Corn Endosperm the loss is 0.165 Cal. per gram or 4.1 per cent; for Wheat Endosperm, 0.250 Cal. per gram or 5.6 per cent; and for "Whole Wheat", 0.412 Cal. per gram or 9.3 per cent. The whole grains, or nearly whole grains, obviously lose more than the endosperm products on account of the low digestibility of the bran.

The calorimetric heat value of the Rolled Oats is highest because of its higher fat content, the "Whole Wheat" is next in order, but because its fat content is lower than that of the Wheat Endosperm, it ranks only a little higher than the latter. The Corn Endosperm is lowest of the four cereal products compared. Only one of the oats preparations was burned in the bomb, because it was assumed that the Precooked Oats would have the same heat value as the regular Rolled Oats. The chemical analyses given in Table I do not quite justify this assumption, although the difference is not great. The "Toasted Whole Wheat" was burned and was found to have almost identically the same heat value as the "Whole Wheat" not toasted. It is not included in Table II, because the data for calculation of the physiological heat values were not available.

For actual value to the body on the basis of these studies the Rolled Oats ranks first, the "Wheat Endosperm" next, and the Toasted Corn Endosperm and "Whole Wheat" tie for third place.

The nutritive value of the different preparations, however, may not follow exactly this order because of differences in *rate of digestibility*. The following paper deals with this factor.

#### SUMMARY

Six different cereal breakfast foods, three from wheat, two from oats and one from corn, all widely used in the United States, have been analyzed, all but one burned in the bomb calorimeter, and their physiological heat values compared with the combustion heat values in four, as a preliminary to a more detailed comparative study of nutritive values.

#### REFERENCES

- Atwater, W. O., *Conn. (Storrs) Exp. Sta. Rep.* 1901.
- Atwater, W. O., and F. G. Benedict, Experiments on the metabolism of matter and energy in the human body. *U. S. Dept. Agr. Off. Exp. Sta. Bull.* 136, 1903.
- Cori, C. F., The fate of sugar in the animal body. I. The rate of absorption of hexoses and pentoses from the intestinal tract. *Jour. Biol. Chem.*, 1925, LXVI, 691.
- Folin, O., and H. Wu, A system of blood analysis. *Jour. Biol. Chem.*, 1919, XXXVIII, 81.
- Greaves, J. E., and C. T. Hirst, The mineral content of grain. *This Journal*, 1921, I, 293.
- Harcourt, R., Breakfast foods; their chemical composition, digestibility and cost. *Jour. Soc. Chem. Ind.*, 1907, XXVI, 240.
- Rehfuss, M. E., A new method of gastric testing, etc. *Amer. Jour. Med. Sci.*, 1914, CXLVII, 848.
- Rehfuss, M. E., O. Bergeim and P. B. Hawk, Gastro-intestinal studies, I, II. *Jour. Amer. Med. Assoc.*, 1914, LXIII, 11, 909.
- Riche, J. A., An improved type of calorimeter to be used with any calorimetric bomb. *Jour. Amer. Chem. Soc.*, 1913, XXXV, 1747.
- Rubner, M., Die Gesetze des Energieverbrauchs bei der Ernährung. F. Deuticke, Leipzig, 1902, 97.
- Woods, C. D., and Harry Snyder, Cereal Breakfast Foods. *U. S. Dept. Agr. Farmer's Bull.* No. 249, 1906.



# THE NUTRITIVE VALUE OF CEREAL BREAKFAST FOODS

## II. DIGESTIBILITY IN VITRO, WITH A STUDY OF METHODS

By

J. S. CARMAN, H. G. SMITH, GLADYS C. HAVENS AND J. R. MURLIN  
(*From the Department of Vital Economics, University of Rochester,  
Rochester N. Y.*)

Received for Publication—June 5, 1929

THREE different studies were made of the digestibility in glassware of the cereal breakfast foods whose analyses and heat values have been described in the preceding paper. In the first only three of the foods were used, namely, the Rolled Oats, the Toasted Corn Endosperm and the Wheat Endosperm.

### FIRST STUDY

The method adopted in this study has, so far as we are aware, not been employed hitherto. It consisted essentially in carrying the same materials through a digestion by salivary amylase, pepsin-HCl, trypsin and diastase, successively.

#### *Method 1. Mouth Insalivation and Successive Digestions on the Same Sample*

Fifty-gram samples of each cereal were weighed into beakers. The wheat and oats products were boiled in 400 and 300 cc. of water, respectively, containing a little salt. The corn product, being already dextrinized by toasting, was not cooked. The cooked cereals were then transferred to weighed beakers and the cooking vessel washed out, the washings being added. Aliquot duplicate samples were next taken of all three cereals and set aside as controls. The rest of the cereal was taken into the mouth and chewed until ready for swallowing and then was expectorated into weighed beakers. The entire quantity thus prepared for salivary digestion was then divided by weighing by difference into 14 nearly equal portions, placed in as many smaller beakers, the volume of each being made up to 78–80 cc. All were incubated together for two hours at body temperature, after which two were heated to stop the action of ptyalin. To all the others HCl and pepsin were now added. Two of these were immediately heated to stop the action of pepsin and the balance incubated for two hours at body temperature, and so on.

Table I exhibits the entire scheme of treatment. In the case of each enzyme there were two control vessels in which digestion was not allowed to proceed and two in which digestion continued for two hours with stirring at half-hour intervals. The amount of digestion of starch by ptyalin, for example, then was determined by the difference in the amount of sugar formed in the filtrates of the digested as compared with the control lots. Sugar was determined by Benedict's (1911) quantitative method and calculated as glucose. As a further check on this method the residual starch left on the filter paper after successive washings to remove sugar was digested in 2.5 per cent HCl under reflux for two and a half hours and the sugar from this digestion determined in the same manner. The amount of sugar found in the filtrate after hydrolysis, expressed as starch, should agree with the difference between the original starch and that found (as sugar) after digestion.

TABLE I  
PARTITION OF EACH CEREAL FOR CONTROLS AND FOR DIGESTION SUCCESSIVELY  
BY DIFFERENT ENZYMES

<i>Samples.</i> 1 2		Remainder of each cereal chewed thoroughly, expectorated into weighed beakers, divided into 14 nearly equal portions (by weighing by difference) as follows:													
Set aside before chewing as controls		3	4	5	6	7	8	9	10	11	12	13	14	15	16
		All digested in incubator 2 hrs. at 38-39°C. Shaken every half hour.													
1 2 3 4		5 6 7 8 9 10 11 12 13 14 15 16													
Boiled 3 and 4 several min. to stop ptyalin. Washed all 3 times by decantation, filtered, sugar determined in filtrate; residue digested with 2.5% HCl and sugar from residual starch determined.		Added 10 cc N · HCl+5 cc pepsin solution.													
		5 6		7 8 9 10 11 12 13 14 15 16											
		Boiled at once to stop pepsin		Incubated 2 hrs. at 38-40°. Shaken every half hour.											
				7 8		9 10 11 12 13 14 15 16									
				Boiled after 2 hrs. incub.		Added 4 cc 2.5 N · NaOH to neutralize acid then 10 cc trypsin-diastrase solution in 0.1 N · NaOH									
						9 10 11 12				13 14 15 16					
						Heated at once to stop trypsin & diastrase.				Incub. 2 hrs. 38-40° then heated.					
						9 10		11 12		13 14		15 16			
						Controls for trypsin		Controls for diastrase		Precip. with trichloracetic		Washed by decantation.			
						9 10 11 12				13 14 15 16					
						Same method for tryptic digestion as for peptic.				Same method for diastase as for salivary amylase.					

In following the proteolytic digestion by pepsin and trypsin, as will be noted in Table I, comparison was made between the samples heated at once after the addition of the enzymes and similar samples heated after digestion for two hours. The undigested protein was then precipitated by means of trichloroacetic acid, the mixture being heated to boiling after standing for two minutes and filtration immediately following while the mixture was still hot. The residues on the filter paper were washed with hot water several times, the filtrate cooled and made up to volume after which nitrogen was determined on an aliquot sample.

This method, while especially satisfactory from the standpoint of economy of time and as a means of imitating the proper order of digestion in the alimentary tract, did not always give satisfactory comparisons. The reasons probably are amongst the following. 1. It is difficult to secure exactly equal salivation in cooked and uncooked cereals and of two different

cooked cereals of wholly different physical characteristics. 2. The division of the cooked and chewed cereal into equal portions by difference by weight is not very exact because of the difficulty of maintaining homogeneity in the suspension during weighing operations. 3. Determination of starch digestion for assay of sugar in the filtrates containing a considerable amount of protein was not wholly satisfactory. For these reasons the results of this first study will not be given in detail. We believe that with suitable precautions, and some slight modifications, the method can be made very serviceable.

The results indicated that the Toasted Corn Endosperm gave the largest percentage of digestion of starch in two hours, the Rolled Oats next, and the Wheat Endosperm third. Peptic digestion, however, was best in the wheat product, second in the oats, and third in the corn product. The already soluble carbohydrate in the toasted corn product was, of course, properly controlled and the same is true of the soluble proteins present in considerable amount in both the corn and the oats products (Harcourt 1907).

## SECOND STUDY

In the second study the difficulties encountered in the first were overcome to a large extent by weighing the individual samples separately and cooking them separately in individual double glass boilers, consisting of two beakers of the same shape. Saliva was collected from several different individuals, mixed, filtered, and added in equal amounts to the several samples. It was desired in this study also to observe the difference due to different lengths of digestion and also the effects of cooking different lengths of time. Consequently the entire number of samples of a single cereal started simultaneously was increased to twenty-four. The order of digestion and the general treatment of the product in each case was similar to the outline already exhibited in Table I, that is to say, peptic digestion followed salivary digestion, tryptic digestion followed peptic digestion, and digestion by pancreatic diastase occurred simultaneously with the tryptic digestion.

### *Method 2*

Samples 1 to 6 were distributed as follows; 1 and 2 for controls, 3 and 4 for salivary digestion one hour, 5 and 6 for salivary digestion two hours. Samples 7 to 12 likewise were distributed as follows: 7 and 8 served as controls, 9 and 10 peptic digestion for one hour, 11 and 12 peptic digestion for two hours. Samples 13 to 18 inclusive were distributed in similar manner for tryptic digestion. Samples 19 to 24 inclusive similarly for pancreatic diastase.

Under favorable conditions, and with plenty of assistance, it is possible to put one cereal of a single cooking through the entire program of digestion in a single day, but as a rule it was

necessary to stop digestion by heating at some intermediate point as, for example, just before pancreatic digestion, and to leave it in this condition over night.

It was found that the tendency of the cereals to form a crust after cooking was a serious obstacle to uniform admixture with the saliva unless the cereals were stirred thoroughly during the process of cooking. As in the first, so in this study, no attempt was made to stop the action of ptyalin by heat in the samples which were to be continued under peptic digestion, but to depend upon the addition of hydrochloric acid for this purpose.

For peptic digestion Merck's powdered pepsin was used in 2 per cent solution. This was added to each of the beakers No. 7 to 24 in sufficient amount to make 0.17 grams of pepsin in the digestive mixture, following the addition of HCl to a concentration of one-tenth normal. Beakers 7 and 8 received the enzyme and then were immediately heated to stop its action. Beakers 9 and 10 were removed from the incubator at the end of one hour and were heated. Beakers 11 and 12 were removed at the end of two hours and heated. Beakers 13 to 24 at the end of two hours received 10 per cent sodium hydroxide in sufficient amount to neutralize hydrochloric acid.

Before addition of pancreatic enzymes, the samples were allowed to stand following neutralization for at least one-half hour to allow for change of reaction due to amphoteric properties of the cereal protein. The reaction was corrected, if necessary, to the neutral point after which sodium carbonate solution was added in sufficient amount to make the mixture, increased in volume, contain 0.3 per cent of the alkali. Fairchild Bros. and Foster's *Extractum pancreatis* was used in 4 per cent solution and a sufficient amount added to each of the beakers 13 to 24 to make 0.4 per cent of the dried extract. Beakers 13 and 14 were immediately heated to serve as controls for tryptic digestion. Beakers 19 and 20 served as controls for diastase digestion. The entire twelve remaining beakers were then placed once more in the incubator and after one hour beakers 15 and 16, 21 and 22, were removed and heated to above 80°. At the end of two hours the remaining four beakers were heated likewise to above 80°.

Separation of the soluble portions from the undigested residue after starch digestion was accomplished by centrifugation. The supernatant fluid was perfectly clear for the wheat preparations and only slightly turbid for the oats preparations. In the pancreatic samples (19 to 24) on the other hand, there was a thin milky suspension due to the small amount of fat set free by proteolytic digestion and not split by lipolytic digestion. This, however, did not interfere with the determinations either of sugar or nitrogen.

Sugar was determined in the supernatant fluids by a modification of Bertrand's (1906) method. The modification consisted of separating the copper oxide by centrifugation rather than by filtration through a Gooch crucible, washing the copper oxide once with distilled water and centrifugation again, after which the oxide was dissolved in the ferric sulfate-sulfuric acid solution. This solution was titrated in the usual manner with permanganate.

In this second study two wheat products and two oats products were used. The first wheat product was the one described in the first study under the name of Wheat Endosperm. The second was the "Whole Wheat" ground to about the same degree of fineness as the Wheat Endosperm preparation.

The second oats preparation was the same commercial brand as the one described in the first study under the name Rolled Oats but was precooked. For this reason it will be described simply as Precooked Oats.

The results of this method of study were again not wholly satisfactory, probably because salivary amylase is not wholly inactivated by heating

to boiling temperature (Gramenitski, 1910; Biedermann, 1914-1916). Boiling had been used to arrest the action of ptyalin immediately after addition of saliva also at the end of one hour and of two hours. We were not aware of this surprising stability of ptyalin at the time this part of the work was done. The second cause of discrepancy probably consisted in the unequal separation of partially digested (that is, soluble) starch from residue by centrifugation. That a considerable amount of soluble starch was present in the supernatant fluid was demonstrated by hydrolysis with hydrochloric acid. The two fractions, reducing sugar before hydrolysis and reducing sugar after hydrolysis, were determined separately.

The determination of sugar by the Bertrand method of supernatant fluids was satisfactory in spite of the small amount of soluble protein present. This was confirmed by a precipitation of the proteins, where they occurred in sufficient quantity, by means of 10 per cent sodium tungstate. Precipitation of proteins for determination of the proteolytic digestions was accomplished, as before, by precipitation with trichloroacetic acid. The washing of the precipitate as in the first study did not always yield good duplicates.

For the several reasons enumerated above, the results of this study will not be reported in detail. They indicated that Wheat Endosperm showed practically the same digestion of starch under salivary amylase at the end of one hour and of two hours as did the Precooked Oa s. The "Whole Wheat" preparation gave similar percentages at both intervals and the Rolled Oats preparation gave the least percentage of digested starch at both intervals. These results were reached by both methods mentioned under the first study; namely, first, direct determination of the total sugar in the supernatant fluid, after hydrolysis and centrifugation, calculated as starch, and second, hydrolysis of the residual starch and subtraction of the resulting sugar, calculated as starch, from the original starch content. Not quite the same order of results was obtained after cooking for one hour as for cooking for fifteen minutes. The results of the proteolytic digestions were a little irregular in that the amount of digestion obtained after one hour of cooking was less in several instances than after fifteen minutes of cooking. The results indicated, however, that the two wheat preparations were about on a par, one with the other, both under peptic and tryptic digestion. The two oats preparations also did not differ widely under peptic digestion but showed a surprisingly larger percentage of digestion under trypsin after cooking for one hour in the Rolled, as compared with the Precooked Oats.



### THIRD STUDY

It was thought best to separate the digestion by different enzymes rather than to use the enzymes in succession on the same samples as in the two previous studies. Cumulative errors in the different stages of digestion would thereby be avoided. In this study also we were interested again in the effect of cooking time upon digestibility as well as in the progress of digestion at different intervals of time. Consequently to follow the effect of different enzymes in succession on the same sample would require too many samples at the start to be readily manageable. As will be seen in the tables which follow, the partial digestion of starch all the way to reducing sugar, as compared with total starch made soluble, was studied. With the proteolytic enzymes the criterion of digestion was the amount of protein made filterable through a given filter paper without reference to whether this protein in solution was in the form of proteoses or final split products.

The third wheat product, "Toasted Whole Wheat" was introduced in the third study. This, like the other cereals is a package product differing from the "Whole Wheat" apparently only in the fact that it was toasted before packing. The chemical analyses, however, show that it contains less protein and slightly more fat. These differences may well be due to the kind of wheat selected.

#### A. SALIVARY DIGESTION

The procedure for study of salivary digestion was as follows: The cereals were cooked in a single boiler for different lengths of time, cooled to body temperature, mixed with saliva, and then subjected to incubation up to a maximum of two hours. In the beginning, only 3 grams of each cereal were used, and at first 10 cc. of saliva. In all cases saliva was collected from only two individuals and these two salivas were mixed in equal proportion. After the preliminary experiments, a large quantity of saliva was collected over a period of several days from the same two subjects, mixed in equal proportion, preserved with chloroform, and placed in the ice box. Thereafter, until the very last experiments of the series, saliva was used from this stock supply, and was therefore uniform in diastatic enzyme and in other qualities.

In the first two series of experiments in the third study the small beakers, which served as single boilers, after cooling to body temperature, were placed in a large incubator room for periods of 1 hour and 2 hours, after which the beakers were heated to boiling temperature to arrest the

ptyalin. The contents of the beakers were then washed out, diluted and filtered or centrifuged, and sugar determined on the filtrate or supernatant fluid.

TABLE II  
AVERAGE PER CENT STARCH DIGESTED COMPLETELY TO SUGAR BY

Digestion for — — — 5 min. After cooking — — 5 min.	5 min.		1 hr.		2 hrs.	
	5 min.	15 min.	5 min.	15 min.	5 min.	15 min.
Wheat Endosperm	19.4	22.7	33.6	32.0	35.0	34.4
Precooked Oats	38.7	34.9	47.6	47.1	52.4	50.3
"Whole Wheat"	23.2	26.8	37.4	40.9	41.0	42.9
"Toasted Wh. Wheat"	30.1	31.1	49.1	52.4	51.9	55.5

Tables II and III illustrate these experiments. The results are averages of from two to four experiments with duplicate determinations for each. The sugar actually formed by action of the ptyalin of saliva is, of course, maltose and not dextrose, for, according to most authorities (Punnett, 1915; Blake, 1916), a very little dextrose appears in the digestion even of soluble starch by animal or vegetable amylases. The advantage of expressing the results in terms of dextrose, however, is that it permits a direct comparison of the starch immediately transformed into sugar with that only partially transformed or changed far enough to pass through filter paper or escape precipitation by tungstate.

TABLE III  
AVERAGE PER CENT STARCH DIGESTED FAR ENOUGH TO PASS THROUGH FILTER PAPER BY

Digestion for — — — 5 min. After cooking — — 5 min.	5 min.		1 hr.		2 hrs.	
	5 min.	15 min.	5 min.	15 min.	5 min.	15 min.
Wheat Endosperm	59.8	70.7	84.1	82.6	85.2	87.6
Precooked Oats	76.8	77.2	82.1	86.0	83.9	88.0
"Whole Wheat"	69.6	67.6	87.5	73.8	90.6	85.3
"Toasted Wh. Wheat"	62.5	66.8	88.4	89.8	90.2	89.7

Later it was learned that a smaller amount of saliva sufficed to give a sufficient amount of digestion in a short space of time for accurate measurement, and more accurate temperature control was secured by use of a small incubator.

Referring now to the results of the comparison of different cereals in their behavior to different lengths of time of boiling, we see from the average figures in Table II that when the cereals were cooked 5 minutes

and 15 minutes, so far as the immediate formation of sugar is concerned, the Precooked Oats heads the list followed by the "Toasted Whole Wheat" and "Whole Wheat" and Wheat Endosperm in that order. When, however, the filtrate was hydrolyzed with hydrochloric acid, converting not only maltose but also soluble dextrins to dextrose and then analyzed, it was found (Table III) that all four cereals stand on practically the same footing. The surprising thing in this study is that from one half to nearly four-fifths of the digestion accomplished in 2 hours takes place in the first five minutes. We shall have more to say about the importance of this observation later.

The figures for percentage of starch completely changed into sugar in 5 minutes of digestion, after 15 minutes cooking, as compared with 5 minutes cooking, are raised a little for the Wheat Endosperm, the "Whole Wheat" and the "Toasted Whole Wheat," but not for the Precooked Oats. When digestion goes on for as long as 1 hour or 2 hours there is no advantage in cooking 15 minutes over cooking 5 minutes for the Wheat Endosperm or the Precooked Oats, but there is some advantage for both "Whole Wheat" preparations.

#### *Method 3*

In the course of the second study trial was made of sodium tungstate as a means of precipitation of the soluble proteins in the supernatant fluids. In the third study it occurred to us to use sodium tungstate as a means of precipitation of both protein and non-digested starch. We were encouraged in the belief that this reagent would prove useful as a means of securing protein-free filtrates by the experience of Rumsey (1922) in his study of wheat flours. In the preliminary trials with this method we were led to suspect the stability of saliva to heat and on search of the literature discovered Biedermann's paper, referred to above, confirming this observation. The well-known rapidity of the action of ptyalin also led us to make a further modification providing for the dumping of equal quantities of mixed saliva into the several beakers inside the incubator, simultaneously, so that digestion might be started at the same instant in the several preparations. The same mechanical device providing for the tilting of test tubes containing the saliva, served also for dumping equal quantities of the sodium tungstate into the several beakers simultaneously to stop salivary digestion. This simultaneous starting and stopping of digestion in one operation proved to be in importance second only to the use of sodium tungstate as a precipitant and both improvements together enabled us to secure perfectly satisfactory duplicate determinations in all of the cereals and under all conditions employed with the different enzymes.

The use of sodium tungstate had to be adapted to the reaction and different physical conditions of the cereal breakfast foods and therefore suffered considerable modification from the conditions found most satisfactory by Rumsey. We found 15 per cent sodium tungstate appropriate but at times it was necessary to use alcohol in conjunction with the tungstate to secure a perfectly clear filtrate upon which satisfactory sugar or nitrogen determinations could be made. Also, it was found necessary to change the reactions considerably from that found most favorable by Rumsey. Indeed, each cereal required a different reaction. The use of this method, however, made it possible to employ Benedict's quantitative method for sugar, which is considerably more rapid than the method of Bertrand and just as accurate in water-clear filtrates with no interfering substances present.

Tables IV and V give the results of simultaneous experiments after cooking 30 minutes and 1 hour, respectively, where the four cereals were used in parallel lots. The digestion for 15 minutes and 1 hour, however, were run on separate days and really constitute separate and independent experiments.

TABLE IV  
AVERAGE PER CENT STARCH TRANSFORMED TO SUGAR BY

Digestion for — — — After cooking — — —	15 min.		1 hr.	
	30 min.	1 hr.	30 min.	1 hr.
Wheat Endosperm	17.4	25.9	22.4	29.8
Precooked Oats	38.6	38.5	44.0	45.8
"Whole Wheat"	24.7	38.7	31.6	42.0
"Toasted Whole Wheat"	44.1	43.6	47.5	51.1

By cooking 30 minutes, as may be seen in Table IV the digestibility of the starch in Wheat Endosperm is 17.4 per cent, in Precooked Oats it is 38.6 per cent, in "Whole Wheat" 24.7 per cent, and in "Toasted Whole Wheat" 44.1 per cent. All of these figures are for digestion only 15 minutes.

In the same summary table are presented, the results after cooking one hour. Digestibility in 15 minutes' exposure to saliva is raised for Wheat Endosperm from 17.4 per cent after cooking 30 minutes to 25.9 per cent after cooking one hour, a relative increase of 50 per cent. The digestibility of the oats product is scarcely improved. The same is true of the "Toasted Whole Wheat" but with the "Whole Wheat" digestibility is raised from 24.7 per cent after cooking 30 minutes to 38.7 per cent after cooking 1 hour, or again, a relative increase of 50 per cent.

In Table IV are presented also the data for sugar formation after 1 hour of digestion. It may be noticed that carrying digestion only 15 minutes yields fully four-fifths of the quantity of sugar produced in one hour, whether cooking is for 30 minutes or one hour. In other words, the amount of sugar produced by ptyalin is not at all proportional to the time of exposure. Biedermann (1914-1916) has called attention to the almost "explosive" character of the action of saliva on starch paste. The action on the starch of cooked cereals is not exactly *explosive*, but may be described as *very rapid*.

Much less benefit of cooking 1 hour as compared with 30 minutes is seen when we lay side by side the results of digestion for one hour. The Wheat Endosperm gains 7.4 per cent, the Precooked Oats 1.8 per cent,

the "Whole Wheat" 10.4 per cent, and the "Toasted Whole Wheat" 3.6 per cent. The two precooked foods gain less than the raw foods.

TABLE V  
AVERAGE PER CENT STARCH TRANSFORMED FAR ENOUGH TO ESCAPE  
PRECIPITATION WITH TUNGSTATE

Digestion for After cooking	— — —			1 hr.
	—	—	—	30 min. 1 hr.
Wheat Endosperm			77.2	80.9
Precooked Oats			76.1	80.5
"Whole Wheat"			77.7	84.3
"Toasted Whole Wheat"			85.4	90.7

On the other hand, the percentage of total starch transformed far enough to escape precipitation with sodium tungstate and existing as water-clear filtrate is raised to about the same extent by cooking one hour, as compared with cooking 30 minutes, for all four cereals (Table V). Precooking, therefore, affects only the digestion to sugar.

We may summarize all the results on salivary digestion briefly in the following statements: After cooking 5 minutes the starch and dextrins of all of the cereal breakfast foods studied are transformed to maltose to the extent of 20 to 40 per cent after exposure to saliva at body temperature for only 5 minutes. They are digested far enough to be in perfect solution to the extent of 60 to 75 per cent by exposure for only 5 minutes. After cooking for only 5 minutes the starches and dextrins of all of the cereal breakfast foods studied are transformed to maltose by exposure to saliva for 1 hour to the extent of 35 to 50 per cent. They are digested far enough to pass through filter paper and form a perfect solution after the same length of digestion to the extent of 82 to 88 per cent. These figures are but little changed by exposure to saliva for 2 hours. In other words, the digestion of starches and dextrins in the cereal breakfast foods would be nearly complete at the end of 1 hour's exposure to saliva.

Using 5 to 10 cc. of saliva to 3 grams of cereal represents very thorough insalivation, such as would take place in prolonged chewing of the cereal and digestion in the mouth. Saliva acts so rapidly that thorough mastication is of more importance than longer cooking (cf. Day, 1908). Boiling 30 minutes instead of 15 minutes improved digestibility only 3 or 4 per cent in absolute terms. Relatively, however, this amounts to from 10 to 30 per cent. One reason for this is the tendency of the cereals to clump or gelatinize and stick together while actively boiling, as will be emphasized

more especially under the head of tryptic digestion. Boiling for one hour as compared with boiling for 30 minutes, improves complete digestibility for 15 minutes' exposure more than 50 per cent, in relative terms, for Wheat Endosperm and "Whole Wheat," the two cereals which are not precooked, but not at all for the Precooked Oats and the "Toasted Whole Wheat" the two which are precooked. It improves partial digestibility; that is, the conversion of starches and dextrins to the point where they escape precipitation with tungstate to the extent of only 5 to 10 per cent relative, for any cereal.

In general it is clear from this study on salivary digestion that cooking any of the cereals for 5 minutes gives a very good start to the utilization of the energy contained in cereal foods *in the form of starch*. Previous dextrinization facilitates this earlier transformation enormously.

## B. PEPTIC DIGESTION

The general procedure for the study of the action of pepsin on the cereal breakfast foods was as follows: 5 grams of each cereal were weighed out, placed in a small beaker with 80 cubic centimeters of water and boiled over a hot plate for the requisite length of time, after which the cereal was cooled to body temperature, 20 cc. of N/10 HCl added, and the volume made up to 100 cc. The beakers were then set in the incubator in such a position that eight cubic centimeters of pepsin solution could be dumped simultaneously into all. Incubation then proceeded for the proper length of time at the expiration of which digestion was stopped in all the beakers by dumping 25 cc. of 15 per cent sodium tungstate solution simultaneously from test tubes into all the beakers. After filtration, nitrogen determinations were made on the filtrates.

A considerable amount of soluble nitrogen already exists in cereals, particularly cereals that have been cooked. Hence it was necessary to determine how much nitrogen could be obtained in filtrates from sodium tungstate precipitation of the cereals after cooking but without digestion. After deducting the soluble nitrogen present in the pepsin solution itself there remained, after boiling 15 minutes, 14.1 per cent soluble nitrogen in Wheat Endosperm, 12.3 per cent in Precooked Oats, 14.3 per cent in the "Whole Wheat" and 18.1 per cent in the "Toasted Whole Wheat." The results were the same after boiling 30 minutes and 1 hour except in the case of the Wheat Endosperm which showed an increase of soluble nitrogen after cooking for 1 hour. Deduction of these amounts expressed as percentages of the total nitrogen present in the cereals has been made in the following tables.

A preliminary study of peptic digestion showed that very little additional soluble nitrogen could be obtained after digestion for only 5 minutes, hence the smallest time used for digestion in this section is 15 minutes. Also, since digestion was found to be as small as 5 per cent in some of the determinations, after cooking 15 minutes, it was deemed unwise to attempt quantitative determinations after cooking for less than 15 minutes. In Table VI are presented the results of digestion for 15 minutes and 2 hours, following 15 minutes, 30 minutes and 1 hour of cooking. The order of digestibility was practically the same in all of the individual

tests averaged (six for each cereal) namely; the Wheat Endosperm first; the "Whole Wheat" second, in all but one test; Precooked Oats third, in all but one; "Toasted Whole Wheat" fourth, in all but one. It is clear that the cereal preparation which contains no bran and no germ of the grain and is not precooked in any way is the most digestible in pepsin-hydrochloric acid. This is uniform for all the different cooking times.

TABLE VI  
PER CENT PROTEIN MADE NON-PRECIPTABLE BY SODIUM TUNGSTATE BY

Digestion for — — — After cooking — — —	15 min.		2 hrs.		1 hr.
	15 min.	30 min.	15 min.	30 min.	
Wheat Endosperm	13.3	16.0	22.9	26.1	38.0
Precooked Oats	7.5	8.2	13.1	13.7	24.1
"Whole Wheat"	7.3	10.1	13.1	13.7	24.2
"Toasted Whole Wheat"	7.2	6.2	13.6	12.8	19.0

The increase in digestibility to 15 minutes exposure after 30 minutes, as compared with 15 minutes, cooking is from 1 to 3 per cent in terms of nitrogen or protein rendered soluble. In relative terms the increase is from 10 per cent in the case of the precooked oats to 20 or 25 per cent in the case of the whole wheat. In the case of the toasted wheat the average digestibility is actually less after cooking 30 minutes than after cooking 15.

We have also in Table VI a direct comparison, after digestion for 2 hours, for the effects of cooking 15 minutes, 30 minutes and 1 hour. The increase in digestibility for 30 minutes as compared with 15 minutes varies from 1.6 per cent absolute in the case of the "Whole Wheat" and the Precooked Oats to 3.2 per cent in the case of the Wheat Endosperm. These percentages again are in terms of protein rendered soluble. The relative increase varies from 5 or 6 per cent in the case of the "Whole Wheat" and Precooked Oats to nearly 15 per cent in the case of the Wheat Endosperm. Again we find that the Toasted Wheat is less digestible after 30 minutes of cooking than after 15 minutes. The reason for this, as was noted under salivary digestion, is the tendency of this product to clump with longer cooking (see Tryptic Digestion, p. 104). Skin formation, which is so troublesome with the double boiler, does not take place in the active bubbling which prevails in a single boiler. Cooking for 1 hour increases the digestibility, as compared with cooking for 30 minutes, from 6 to 12 per cent in terms of proteins rendered soluble, or, in relative terms, from about 40 per cent in the case of the Wheat Endosperm to about 80 per cent in the case of the "Whole Wheat" and Precooked Oats.

The clumping, just noted, does not seem to interfere with the more prolonged digestion so much as it does with the shorter digestion. We shall see that the results of clumping in tryptic digestion are somewhat different. The order of digestibility after cooking from 30 minutes to 1 hour is the same as already noted for cooking 15 minutes. In fact, in 5 out of 6 tests, averaged in Table VI, the order of digestibility, after cooking 1 hour, is, Wheat Endosperm first, "Whole Wheat" second, Precooked Oats third, and "Toasted Whole Wheat" fourth.

The conclusions which may be safely inferred from the results of peptic digestion are as follows: Cooking for 30 minutes increases the digestibility over cooking 15 minutes in all of the cereals except the Toasted Wheat. The maximum relative increase which occurs in the case of the "Whole Wheat" is about  $1/3$ ; in the case of the Wheat Endosperm it is about  $1/4$ ; and in the other cereals less. Cooking for 1 hour increases the digestibility over cooking 15 minutes and over cooking 30 minutes of all of the cereals. The maximum relative increase by cooking 1 hour as compared with 15 minutes is in the neighborhood of 80 per cent in the case of the "Whole Wheat" and in the neighborhood of 60 per cent in the case of the Wheat Endosperm. The maximum relative increase after cooking 1 hour as compared with 30 minutes is in the neighborhood of 75 per cent for the "Whole Wheat" and about 45 per cent in the case of the Wheat Endosperm and the "Toasted Whole Wheat."

The proteins and other nitrogenous compounds of the cereal grains found in the bran and the germ are less digestible than the proteins found in the endosperm of the grain (Holmes, 1919). This has often been observed in the case of breads made from whole grain and from highly milled grain (Snyder, 1901-1905; Rubner, 1883, 1916; Food (War) Committee of Great Britain, 1918). This is probably the reason why the Wheat Endosperm, being entirely free of bran and wheat germ, is the most digestible of the four cereals studied. It also probably accounts for the positions of the other cereals in the order of digestibility. Nothing need be said here about the relative biological values of the proteins contained in the germ and endosperm.

### C. TRYPTIC DIGESTION

The same general method was followed for studying the effects of trypsin as for studying the effect of pepsin. Five grams of each cereal were weighed out, placed in a small beaker, and boiled with 80 cc. of water for the requisite length of time. After cooling, sodium carbonate was added so as to make the mixture contain 0.3 per cent of this salt. The beakers were then placed in the incubator and 8 cc. of a 3 per cent trypsin solution were dumped simultaneously from test tubes into all the beakers. Digestion then continued for the requisite lengths of time at body



temperature, at the expiration of which digestion was stopped by simultaneously dumping sodium tungstate from test tubes into the beakers. Adjustment of the reaction to a different end point for each cereal and addition of an equal volume of 95 per cent alcohol was found necessary to produce complete precipitation. Filtration and determination of the nitrogen found in the water-clear filtrate then followed.

Partly for lack of time, and in order to concentrate attention upon the comparison between different times of cooking, and partly, also, because less error is involved in a longer period of digestion than in a short period, a two-hour period was selected uniformly for the different tests. Tests were made with four different times of cooking, namely, 5 minutes, 15 minutes, 30 minutes, and 1 hour. For reasons which will be explained presently, additional tests, after cooking 1 hour under a reflux condenser, were made. The results of four experiments with duplicate determinations for each are summarized in Table VII. The general order of digestibility for these tests is as follows; the Wheat Endosperm first, the "Whole Wheat" second, the "Toasted Whole Wheat" third, and Precooked Oats fourth. There is just one exception to this order, namely, that with 5 minutes cooking the precooked oats stands ahead of the whole wheats.

TABLE VII  
AVERAGE PER CENT PROTEIN DIGESTED BY TRYPSIN IN 2 HRS. AFTER

Cooking — — 5 min.	*	15 min.	30 min.	1 hr.	1 hr. with condenser	
Wheat Endosperm	61.4	76.6	81.5	72.6	66.3	81.5
Precooked Oats	55.2	55.9	54.1	59.4	55.1	60.6
"Whole Wheat"	54.7	61.5	68.8	67.1	59.6	69.4
"Toasted Whole Wheat"	52.2	63.8	68.7	61.2	59.2	61.3
Wheat Endosperm Toasted	62.5					

\* Digestion with diastase and trypsin together.

It is noteworthy that after cooking 15 minutes digestibility is greater than after cooking 30 minutes in all except one cereal, namely, the Precooked Oats. It is still more noteworthy that digestibility is reduced still further by cooking 1 hour as compared with cooking 15 minutes. This also applies to all of the cereals except the Precooked Oats. Digestibility, after cooking 15 minutes as compared with cooking 5 minutes, however, is greater in all of the cereals except the Precooked Oats.

It was quite obvious from direct inspection of the beakers that the difficulty after cooking the longer times in single boilers was the tendency

of the cereals to form lumps. The Precooked Oats, being of coarser grain, did not show this tendency so much as the other cereals. That this was the real cause is proved by the experience with the condenser. The cause of clumping is the too rapid evaporation of water when the single boiler is used and active boiling is kept up continuously. The simplest remedy is to provide an air condenser so that water driven off in the form of vapor will drip back into the cooking vessel as water. Small Erlenmeyer flasks were substituted for beakers, and into the neck of each flask was fitted a rubber stopper with an upright glass tube about  $\frac{1}{4}$  inch in diameter and about four feet in length. These tubes were clamped in position from a shelf directly above the hot plate on which the cooking was done. This arrangement permitted condensation of the vapors rapidly enough to prevent clumping of the cereals.

The effect on digestibility is shown by comparison of the last column in Table VII with the next to last. The digestibility of all of the cereals is improved. In all except the "Toasted Whole Wheat" the digestibility is raised to the same level, or higher, as attained after cooking only 15 minutes in the usual manner.

Attention may be called to the last figure in the table, namely, the effect of toasting on digestibility with trypsin. Samples of the Wheat Endosperm product were toasted in a baking oven until a light brown color was obtained, very similar to that which prevails in the "Toasted Whole Wheat." In contrast with the effects of toasting upon digestibility of starches and dextrans, we see that toasting does not affect digestibility of proteins; for the average figure, namely, 62.5 per cent obtained after toasting is substantially the same as obtained, 61.4 per cent, before toasting. Attention may be called also to the extra vertical line of figures shown in Table VII under "Cooking 5 minutes." These figures represent averages of two determinations made upon the digestibility of protein under trypsin acting simultaneously with diastase. Correction has been made for the effects of trypsin on the proteins of diastase itself.

The evidence from these few determinations is that trypsin acting simultaneously with a starch-splitting enzyme produces its proteolytic effect more rapidly than when acting alone. We shall see in a moment that trypsin reciprocally favors the action of diastase.

The conclusion under this section may be stated briefly as follows: Cooking for 15 minutes in a single boiler increases digestibility by trypsin over cooking 5 minutes in all cases except the Precooked Oats. The maximum relative increase in digestibility is in the neighborhood of 30 per

cent in the case of the Wheat Endosperm. Cooking in a single boiler for longer than 15 minutes decreases digestibility because of the formation of lumps produced by too rapid evaporation of water. This may be prevented by using a simple air condenser, thus restoring digestibility to its maximum obtained by 15 minutes of simple boiling. There is no advantage, however, in digestibility obtained by cooking up to 1 hour, even with a condenser, over cooking 15 minutes. Simple toasting does not increase the digestibility of the Wheat Endosperm under the action of trypsin as it does under the action of saliva. Simultaneous action of diastase with trypsin facilitates the proteolytic action of the latter ferment probably because the proteins are set free by simultaneous digestion of starch more rapidly than otherwise.

#### D. ACTION OF MALT DIASTASE

In order to study the isolated effect of diastase uncomplicated by the action of any other enzyme, the diastase of malt was selected mainly because it is available commercially in purer form than any other diastase. Its action is, in general, similar to that of pancreatic diastase, but pancreatic diastase entirely free of trypsin is difficult to obtain.

The main consideration in studying the effects of another starch-splitting enzyme, in addition to the ptyalin of saliva, is that normally, in the intestine, a diastatic enzyme (amylase of the pancreatic juice) is active simultaneously with trypsin. It was desirable to determine whether this simultaneous action of trypsin and diastase would produce more rapid formation of sugar, particularly after a brief period of cooking, than action of diastase alone.

Only one complete set of experiments was carried through on this plan (Table VIII) but they are sufficient to prove quite conclusively, because the results are concordant, that the simultaneous action of the two enzymes is much more favorable than was obtained with diastase

TABLE VIII  
EFFECT OF DIASTASE ON STARCH OF 5 GRAMS CEREAL AFTER COOKING 5 MIN.

	Per cent starch digested to sugar in 2 hrs.		Per cent starch rendered soluble in 2 hrs.	
	Diastase alone	Diastase with trypsin	Diastase alone	Diastase with trypsin
Wheat Endosperm	24.8	41.5	89.4	89.4
Precooked Oats	31.9	33.5	75.3	74.7
"Whole Wheat"	28.3	41.2	84.6	84.2
"Toasted Whole Wheat"	27.9	41.6	87.5	87.1

alone, or indeed with saliva alone. The largest effect shown in Table VIII is with the Wheat Endosperm, as would be expected from the fact, already demonstrated in previous sections, namely, that this cereal is the most digestible from the standpoint of its protein content. Rendering the proteins soluble by digestion obviously makes the starch more available to the diastatic enzyme.

The next largest effect occurs in the case of the "Toasted Whole Wheat," which is nearly the same as the effect with the "Whole Wheat," but the effect with Precooked Oats is very slight owing probably to its relatively slow digestibility under the action of trypsin. It should be noted that all of these tests were conducted on the cereals cooked only 5 minutes.

There is practically no effect of the simultaneous action of the two enzymes as compared with diastase alone when we consider the total sugar obtainable from the filtrate after hydrolysis. In other words, it would appear that the favorable action of trypsin has to do with the immediate formation of sugar rather than the formation of soluble dextrans.

Table VIII is already so brief that it need not be summarized further. The conclusion from this section of our study can be stated in very few words. Malt diastase transforms the starches and dextrans of the breakfast cereals into maltose very rapidly; much more rapidly, however, when trypsin is acting simultaneously with the diastase. Cooking 5 minutes is sufficient to insure digestion of the starch to the extent of 75 to 90 per cent in 2 hours.

#### DISCUSSION

In her excellent study of the digestibility of different starches as affected by cooking, Day (1908) reviews the literature of starch chemistry and finds that three substances are present in raw starch grains, which are designated by her, according to the color they give with iodine, blue amylose, red amylose and rose amylose. Blue amylose constitutes 90 per cent or more of the inside of the cereal starch grains; it takes up water at 60 to 80°C and forms the sticky, colloidal substance known as starch paste, in which form it is easily digested. Long boiling up to 3 hours does not make it more digestible. Red amylose makes up the outer layer of starch grains. It swells at temperatures necessary for paste formation and breaks on boiling. In either case it does not hinder digestion. Rose amylose constitutes about 10 per cent of the inner starch. It is found only in cereal starches. It digests more slowly than blue or red amylose, but is converted slowly to blue amylose by cooking and this fact alone accounts for the greater digestibility of cereal starches by long cooking.

But there is a fourth substance found in cooked starch, designated as a reverted amyloextrin, by Syniewski, which is more difficult of solution than any of the amyloses in natural starch. It is found especially in the skin of starch paste and renders this much less digestible. If skin is permitted to form in the cooking of cereals the slight advantage gained by longer, over shorter, cooking, is thereby defeated.

These facts brought to light by Day doubtless account for the results on the effects of cooking different lengths of time, as shown in this paper. Even the raw cereals, Wheat Endosperm and "Whole Wheat" whether digested for 5 minutes, 1 hour or 2 hours, did not show very much greater digestibility after boiling 15 minutes than after boiling only five minutes (Table II). The precooked cereals showed even less improvement. In these short cooking times the rose amylose was not changed to blue amylose. Comparing 30 minutes boiling with 1 hour, the digestibility was improved (Table IV) by the longer boiling about 50 per cent for the raw cereals, but not at all for the precooked. Skin formation was prevented in these experiments by stirring. The rose amylose must have been largely converted by 1 hour of active boiling and no reversion product was formed. Moreover, the cellulose cell walls in the raw cereals were broken down thereby liberating more starch to the action of the salivary amylase.

Our results with the sodium tungstate precipitation, however, reveal the interesting fact that the preliminary digestion to soluble dextrins (not to sugar) is improved to about the same degree (only 3 to 7 per cent) in raw and precooked cereals by boiling one hour over boiling 30 minutes. Since Langworthy and Deuel (1920) have shown that raw corn and wheat starches are completely digested in the human alimentary tract, we agree with Day that thorough salivation of cereal starches is probably much more important than long cooking, so far as starch digestion is concerned.

Only wheat and oats cereals not previously cooked to any extent have been compared in this series of experiments. The results indicate (second study p. 94) that the starch of Wheat Endosperm and of "Whole Wheat" both is more digestible than oats starch in the whole grain. This is in agreement with Woods and Snyder (1906) in their summary of many studies made at different experiment stations on digestibility of different cereals in the human alimentary tract. They find that the three cereals wheat, oats and corn, stand in the order named as to digestibility. There is more reason then for precooking, steaming or toasting, corn and oats than wheat.

When we consider the digestibility of protein in the cereal breakfast foods we find a different order of facts. The protein of wheat endosperm is more digestible than the protein of whole oats whether pre-cooked or not. This applies to all different cooking times and periods of digestion tried. It is true of tryptic digestion as well as of peptic. The results also are in accord with all of the studies reported in the literature on the digestibility of white (endosperm) flour breads as compared with "whole wheat" or Graham breads. They indicate further that the low coefficient of digestibility of whole grain products is not due to greater elimination of metabolic nitrogen from the alimentary tract.

The improvement in digestibility by longer cooking (up to 1 hour) is greater for the protein than for the starch of the cereal breakfast foods. This applies particularly to the whole grain products. The reason doubtless lies in the destruction of cellulose walls especially of the bran. Clumping of the cereal which is so apt to occur in single boilers interferes greatly with this breakdown of the cells.

This study has not been concerned with the digestibility of the small amount of fat in the cereal breakfast foods nor with the optimum conditions for activity of the various enzymes employed. Every effort was made to have the conditions the same in simultaneous tests with the different cereals.

#### SUMMARY AND CONCLUSIONS

1. Six different cereal breakfast foods, all very widely used, have been compared as to their digestibility by salivary amylase, pepsin-HCl, trypsin and malt diastase.
2. Three different methods of study have been used and are described.
3. Only the results of the third method are given in detail. This method made use of sodium tungstate for precipitation of undigested residues. Clear filtrates were obtained and consequently satisfactory duplicate determinations of the stage of digestion reached. A dumping mechanism was employed for starting and stopping the digestions simultaneously for the different cereals.
4. The effects of different cooking times were closely studied.
5. The breakfast foods studied included three of the precooked variety and three previously uncooked.
6. Precooking affects favorably only the digestion of starch.
7. Cooking (boiling) improves the digestibility of the starch less than the digestibility of the protein.

8. Clumping of the cereal in open boiling retards digestion considerably and protein digestion more than starch digestion.

9. The simultaneous action of a proteolytic enzyme (trypsin) and a diastase produces much more rapid digestion of starch and protein than the diastase or trypsin acting alone.

#### REFERENCES

- Benedict, S. R., The detection and estimation of glucose in urine. *Jour. Amer. Med. Assoc.*, 1911, LVII, 1193.
- Bertrand, G., Le dosage des sucres reducteurs. *Bull de la Soc. Chim. de Paris*, 1906, XXXV, 1285.
- Biedermann, W., Fermentstudien, I. Das Speichelferment, *Fermentforsch.*, 1914-16, I, 385.
- Blake, J. C., On the digestibility of bread, I. Salivary digestion. *Jour. Amer. Chem. Soc.*, 1916, XXXVIII, 1245.
- Day, Edna D., Digestibility of starch of different sorts as affected by cooking. *U. S. Dept. Agr. Off. Exp. Sta. Bull.* No. 202, 1908.
- Food (War) Committee, Roy. Soc. Gr. Britain. On the digestibility of bread, 1918.
- Gramenitski, M. J., Der Einfluss verschiedener Temperaturen auf die Fermente und die Regeneration fermentiver Eigenschaften. *Zeitschr. Physiol. Chem.*, 1910, LXIX, 286.
- Harcourt, R., Breakfast foods; their chemical composition, digestibility and cost. *Jour. Soc. Chem. Ind.*, 1907, XXVI, 240.
- Holmes, A. D., Experiments on the digestibility of wheat bran in a diet without wheat flour. *U. S. Dept. Agr. Bull.* No. 751, 1919.
- Langworthy, C. F. and H. J. Deuel, Jr., Digestibility of raw corn, potato and wheat starches. *Jour. Biol. Chem.*, 1920, XLII, 27.
- Punnett, P. W., A study of the products of the action of different amylases—Is monosaccharide formed in the digestion of soluble starch? Dissertation, Columbia University, 1915.
- Rubner, M., Ueber den Werth der Weizenkleie für die Ernährung des Menschen. *Zeitschr. f. Biol.*, 1883, XIX, 45.
- Rubner, M., Die Verdaulichkeit von Weizenbrot. *Arch. f. Physiol.*, 1916, 61.
- Rumsey, L. A., The diastatic enzymes of wheat flour and their relation to flour strength. *Amer. Inst. Baking, Bull.* 8, 1922.

NOVEMBER, 1929

ISOLATION OF NARROW SPECTRAL REGIONS  
BY SELECTIVE ABSORPTION

BY LOYD A. JONES

*(Communication No. 386 from the Kodak Research  
Laboratories Rochester, N. Y.)*

Received for Publication—June 10, 1929

THERE are many problems in the fields of physics, chemistry, biology, physiology, etc., which require the isolation of fairly narrow spectral regions in order that the effects produced by radiation of various wave-lengths may be determined. Obviously, the most elegant solution of the problem is the use of some type of dispersing instrument such as a monochromatic illuminator which will deliver radiation of high spectral purity. In many cases, however, it is not feasible to employ this method because of the high cost of such equipment or because of the impossibility of obtaining the required radiation intensity. For the isolation of relatively narrow spectral regions it is sometimes necessary, therefore, to use light filters made up of selectively absorbing materials. For this purpose there are many materials available, among which may be mentioned solutions, glasses, dyed gelatin, etc. Obviously, the particular method and materials used will be governed largely by the requirements of the problem.

The filters described in this paper were developed at the request of Dr. Ethel M. Luce Clausen who is investigating the action of radiation in biological experiments with the rat.\* That the results might be based on an average of a large number of observations, and therefore be most reliable, this work involved the use of many of these animals. When many of these animals are to be subjected to radiation treatment, it is desirable that several be exposed at the same time. This necessitates the illumination of a relatively large area. Moreover it is desirable that the radiation intensity incident on the animals be relatively high so that

\* See following paper.



the period of treatment need not be unduly prolonged. Since the treatment of a given group of animals may extend over a period of several months, it is obviously desirable that the filters be as stable as possible to avoid the necessity of constantly rechecking and making new filters.

The possibility of using a monochromatic illuminator for this purpose was considered but it appeared that the requirements of area to be illuminated and radiation intensity desired could not be met without the construction of a very special instrument which would be almost prohibitive from the standpoint of cost. A careful survey of the selectively absorbing materials available was therefore made and the filters described in the following pages were constructed.

In searching for suitable selectively absorbing materials, our attention naturally turned first to organic dyes because they are available in an almost endless variety and may be conveniently utilized in the form of dyed gelatin. The entire group of Wratten light filters was examined and the spectrophotometric absorption curves of a large number of dyes, other than those used in the manufacture of Wratten light filters, were studied. With the dyed gelatin already available it was found possible to isolate the red, green, and blue regions of the visible spectrum, excluding completely the ultra-violet. All three of these dyed gelatin filters, however, transmit freely in the infra-red; and, in fact, no dyes having appreciable absorption in the infra-red region were found.

The source of radiation used by Dr. Clausen in this work, an electric arc trimmed with white flame (therapeutic A) carbons, manufactured by the National Carbon Company, emits relatively large quantities of radiation in the infra-red region. In the study of radiation effects it is desirable to work with narrow spectral regions in order to be sure that the effects observed are actually attributable to some definite wave-length of radiation. It was necessary, therefore, to find some material for removing the infra-red radiation transmitted by the dyed gelatin filters used for the isolation of narrow bands in the visible region. It should be emphasized that in all work of this kind the visual judgment of radiation purity is of little value. Since the eye is sensitive to wave-lengths only within the region between 400 and 700  $m\mu$ , the presence of radiations of shorter or longer wave-lengths is not detectable by visual observation and in many cases workers have been grossly deceived regarding the monochromatic characteristics of the isolating filters as determined by visual observation. Several inorganic compounds were found which, when used in an aqueous solution, absorbed the infra-red region fairly well. Of these, copper sulfate ( $CuSO_4 \cdot 5H_2O$ ) seemed most suitable from the standpoint

of stability, reproducibility, and availability. Aqueous solutions of copper sulfate of various concentrations were, therefore, used as an infra-red absorber, the concentration in any case of course being determined by the permissible absorption at other wave-lengths.

Water absorbs strongly in the infra-red and may be used for absorbing radiation of wave-lengths greater than  $1000\text{ }\mu$ .

At the time this work was initiated the long-wave radiation limit of antirachitic radiation was very vaguely defined, it being contended by some that all radiation of wave-length greater than  $305\text{ }\mu$  was entirely without antirachitic effect, and by others that wave-lengths as great as  $350\text{ }\mu$  possessed a certain degree of potency. It, therefore, seemed desirable to split the region between  $380$  and  $480\text{ }\mu$  into several very narrow bands. Considerable effort was expended in this direction without complete success. The filters numbered 5, 6, 7, and 8 represent the best results. A copper sulfate solution in proper concentration absorbs completely all wave-lengths less than  $300\text{ }\mu$ , while a solution of nickel sulfate ( $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ ) transmits this wave-length freely but absorbs very strongly at  $400\text{ }\mu$ . These two solutions, singly and in combination, are valuable, therefore, for controlling the absorption at various wave-lengths in the ultra-violet region (wave-lengths less than  $400\text{ }\mu$ ).

In constructing the ultra-violet filters two Corning glasses, G986A and G586, were found useful for absorbing the visible region. The former transmits freely at  $300\text{ }\mu$ , while the latter absorbs this wave-length but transmits freely between  $310$  and  $410\text{ }\mu$ . Both of these glasses absorb in the infra-red region to a certain extent. G986A is particularly useful in this respect; it has a maximum transmission at approximately  $700\text{ }\mu$ , the absorption increasing rapidly for longer wave-lengths from this point. These two glasses are, therefore, very valuable in work of this kind. Corning glass G980 transmits freely all wave-lengths greater than  $250\text{ }\mu$  throughout the ultra-violet, visible, and infra-red. This glass was found very useful, therefore, in the construction of a tray to hold the liquid components of the filters for the isolation of the ultra-violet bands. When it is not necessary to transmit the ultra-violet, a satisfactory tray for holding the liquid components can be made by using ordinary white optical glass. This term "white optical glass" refers to a colorless, transparent, optical glass such as is used in the manufacture of lenses, prisms, etc. The various component materials used in the construction of the filters may be summarized as follows:

Distilled water,  $\text{H}_2\text{O}$

Aqueous solution of copper sulfate crystals,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

Aqueous solution of nickel sulfate crystals,  $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$

Glass, Corning No. G986A

Glass, Corning No. G586

Glass, Corning No. G980A

Dyed gelatin, Wratten filter No. 88A

Dyed gelatin, Wratten filter No. 25

Dyed gelatin, Wratten filter No. 61

Dyed gelatin, Wratten filter No. 49.

The construction of a suitable container to hold the necessary liquid components was given careful consideration. Past experience has shown that the use of a liquid component in light filters is usually very troublesome since it is difficult to make a container which will not leak when used over long periods of time. The final solution in this case was found in the form of a shallow tray, the bottom of which is composed of six glass plates set into rectangular openings. Constructional details of this tray and drawings illustrating it have been given by Dr. Clausen.<sup>1</sup> The dimensions of the tray,  $19\frac{1}{2}$  inches long by  $11\frac{1}{2}$  inches wide, were adjusted to conform to the irradiation equipment already available. The glass plates forming the bottom of this tray are  $5\frac{1}{2}$  inches by  $4\frac{5}{8}$  inches and when mounted have a clear effective area of 5 inches by  $4\frac{1}{4}$  inches. The total transmitting area of the tray is therefore approximately 120 square inches. This tray sits on top of the irradiation housing, the source of radiation being placed immediately above, and the animals to be irradiated directly under, the tray in a light-tight enclosure. This tray therefore constitutes a means of inserting the glass elements required, the liquid component being held within the tray. Gelatin components are placed beneath the tray so that they are protected from the direct radiation from the source and are thus prevented from heating and injury by exposure to the hot particles which fall from the arc during operation. These gelatin filters are mounted in a wooden frame, also arranged in the form of six rectangular sections similar to a window sash. The openings in this frame correspond to the openings in the tray. This wooden frame, carrying the gelatin film, slides into a horizontal slot just under the tray and when in position completely closes the housing so that no stray light may enter. The glass plates are held in position by means of rectangular metal frames attached to the bottom of the tray with screws. In assembling the tray a pure unvulcanized rubber gasket is first placed in contact with the metal of the tray itself. The glass plate is then laid in position and a second gasket of pure unvulcanized rubber is

<sup>1</sup> The Use of Isolated Radiation in Experiments with the Rat. The Effect of Infra-red Radiation on the Growth of the Rachitic Rat. *This Journal*, 1929, II, 125.

laid on the glass. The metal frame is then attached and the screws holding it in position are drawn down gradually and uniformly on all sides. These holding screws are placed close together so that the pressure may be applied uniformly all around the glass plate. As pressure is applied, the gum rubber is compressed and forms a permanent, liquid-tight joint. During use this material becomes heated to a relatively high temperature and it appears that a certain amount of vulcanization takes place, thus forming an excellent, liquid-tight joint. In all cases, except that of the filter used for isolating a band in the infra-red, some component, either liquid or glass, must be used to absorb the infra-red region. It is obvious that the filter will become rather warm because of the absorption of this long-wave radiation. As a matter of fact, the copper sulfate solution rises to a high temperature and evaporation takes place fairly rapidly. After a solution has been used for some time its volume may be appreciably reduced and hence its concentration increased. It is necessary, therefore, to add distilled water in order to maintain the desired concentration.

When making the spectrophotometric measurements on the various selectively absorbing components in work of this kind, we find it necessary to include a rather great wave-length range. We know that the source is emitting to a certain extent radiations of all wave-lengths from two hundred to several thousand millimicrons. A 2-cm. layer of water is, however, included as one of the components of all filters. This material absorbs almost completely radiations of wave-length greater than 1200 and hence it is necessary, for the purposes of this investigation, actually to make spectrophotometric measurements only in the region between 200 and 1200  $m\mu$ . Since the visible region embraces only wave-lengths from 400  $m\mu$  to 700  $m\mu$ , it is obvious that the required measurements cannot be made visually. It is necessary to use photographic methods for the ultra-violet and the shorter portion of the visible spectrum. By using plates especially sensitized to the infra-red, the photographic measurements can be extended to 900  $m\mu$ . For wave-lengths greater than this, radiometric methods must be employed.

In this work four different spectrophotometers were used:

(a) A quartz spectrograph, made by Adam Hilger, with the Hilger spectrophotometric attachment. This covers the wave-length region from 200 to 600  $m\mu$ .

(b) A spectrophotometer of the Hüfner type made also by Adam Hilger. This is a visual instrument covering the wave-length range from 400 to 700  $m\mu$ .

(c) A photographic spectrophotometer covering the wave-length range

from 600 to 900  $m\mu$ . This consists of a multiple prism instrument made by Hans Heele and used with photographic plates sensitized for the wave-length region mentioned.

(d) An infra-red spectrophotometer, made by Adam Hilger, using a thermopile as sensitive element. This was used in the region of wave-lengths greater than 800  $m\mu$ .

For the benefit of those who may not be intimately familiar with spectrophotometric methods it may be worth while to explain very briefly the principle upon which photometric measurements are made. In the first place some means must be employed for dispersing the radiation into its component parts, thus forming a spectrum in which the various wave-lengths are isolated. This dispersed radiation is then separated into two equal parts by some type of beam-splitting device. The selectively absorbing material is placed in the path of one of these beams. The two parts are then brought together and the intensity of the one which has passed through the absorbing material is measured in terms of the other. In this way the absorbing power of the filter in question is determined, wave-length by wave-length.

The absorbing power of a material may be expressed in several ways. One of the most common is to state the percentage transmission, and if  $I_0$  be used to indicate the intensity of the radiation incident upon the selectively absorbing material, and  $I_1$  the intensity transmitted thereby, then the transmission,  $T$ , is given by the expression

$$T = I_1/I_0.$$

For many purposes it is more convenient to express the transmitting or absorbing characteristics of a material in terms of density,  $D$ , where density is defined by the equation

$$D = \log_{10} 1/T.$$

The use of density, as a means of expressing this absorbing power of material, is particularly convenient because of its logarithmic form. If it is desired to determine the resultant density obtained by superposing one absorbing material on another it is necessary only to add the density values at any particular wave-length; if the absorbing power is expressed in transmissions, the two values of transmission must be multiplied together. Moreover, density is directly proportional to concentration in the case of a solution, and also directly proportional to the thickness of the absorbing layer. All the data given in the following pages relating to these filters are given in terms of density. These values are plotted as ordinates against the wave-lengths as abscissae.

The compositions of the filters finally constructed for the isolation of the desired spectral regions are as follows:

*Filter No. 1—Green*

Composition: Corning glass G980A	0.32 cm.
Solution $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ (50%)	2.0 cm.
Wratten gelatin filter No. 40	

Absorption characteristics, spectrophotometric curve Fig. 1, Chart 1.

It will be noted that the composition of this filter is identical with that of No. 8 with the exception of the Wratten filter No. 40. This absorbs completely the ultra-violet radiation and transmits with little disturbance the green band of the No. 8 filter. As a matter of fact, the maximum transmission in the green is somewhat less, but the decrease is relatively small.

*Filter No. 2—Red*

Composition: White optical glass	0.32 cm.
Solution $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (5%)	2.0 cm.
Wratten gelatin filter No. 25	

Absorption characteristics, spectrophotometric curve Fig. 2, Chart 1.

This filter isolates the visible red. The cut on the short wave-length side of the transmission band is due to the Wratten filter No. 25, while the cut on the long wave-length side of the absorption band is due to the copper sulfate solution. It will be noted that there is a slight residual transmission with a maximum at  $1080 \text{ m}\mu$ ; the minimum density, however, is relatively high, being 2.35, which corresponds to a transmission of 0.44 per cent. It is considered that this is so small as to be negligible.

*Filter No. 3—Infra-red*

Composition: Corning glass G986A	0.32 cm.
Water	2.0 cm.
Wratten gelatin filter No. 88A	

Absorption characteristics, spectrophotometric curve Fig. 3, Chart 1.

This filter represents the best that we have been able to do in the isolation of a narrow band in the infra-red. The cut on the short wave-length side is due to the gelatin component, Wratten No. 88A, while that on the long wave-length side is due to a combination of the absorption of Corning glass G986A and the 2-cm. layer of water.

*Filter No. 4—Blue*

Composition: White optical glass	0.32 cm.
Solution $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (5%)	2.0 cm.
Wratten gelatin filter No. 49	

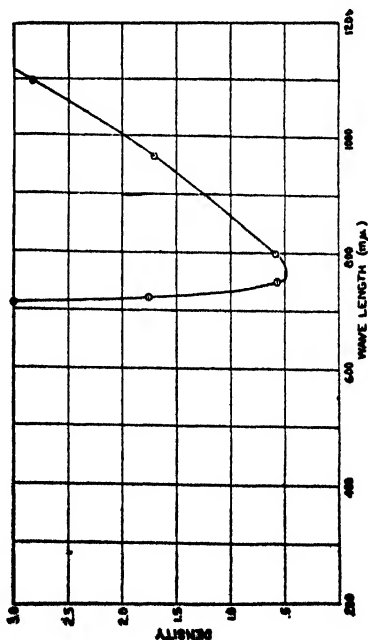
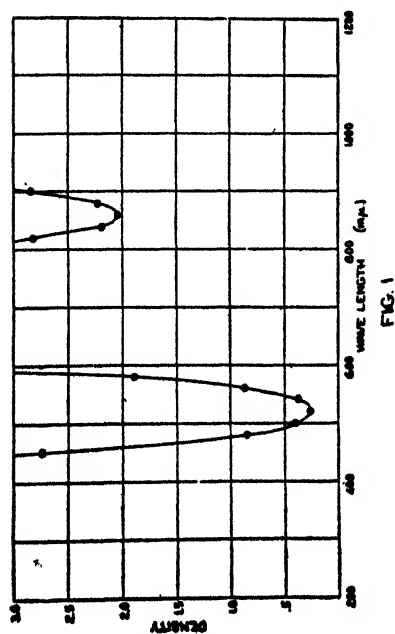
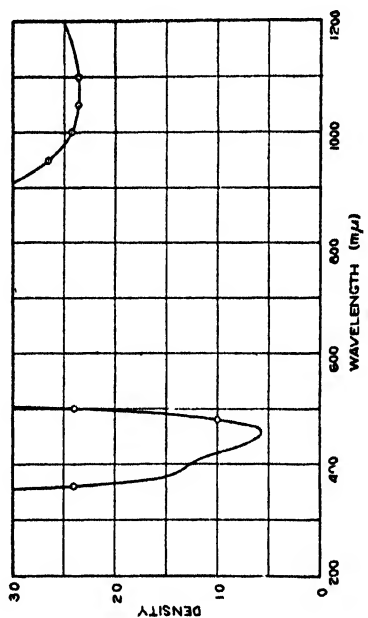
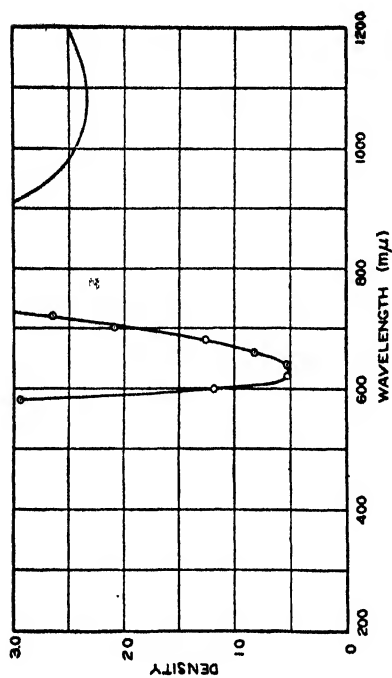


CHART 1. Spectrophotometric absorption curves for filters Nos. 1, 2, 3, 4.

Absorption characteristics, spectrophotometric curve Fig. 4, Chart 1.

This filter isolates the blue region of the visible spectrum, including a little of the near ultra-violet. Again there is a slight residual transmission in the infra-red which is considered negligible.

*Filter No. 5—Ultra-violet*

Composition: Corning glass G986A	0.32 cm.
Solution $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ (50%)	2.0 cm.
Solution $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1%)	

Absorption characteristics, spectrophotometric curve Fig. 5, Chart 2.

This filter isolates a band in the ultra-violet between the limits 270 and 355  $\mu$  with a maximum transmission at 315  $\mu$ . This maximum transmission is, however, relatively low, being approximately 19 per cent. This filter, therefore, although very satisfactory from its position in the spectrum, cuts down the intensity of the radiation more than is desirable. The limit on the short wave-length side of the transmission band is due to the copper sulfate solution, while the limit on the long wave-length side is set by the nickel sulfate solution. The Corning glass G986A serves to absorb the visible radiation and also, when combined with the copper sulfate solution, completely eliminates the infra-red radiation. This filter, therefore, gives a relatively homogeneous band of radiation undiluted by residuals of any other wave-length.

*Filter No. 6—Ultra-violet*

Composition: Corning glass G586	0.32 cm.
Solution $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ (20%)	2.0 cm.
Solution $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (5%)	

Absorption characteristics, spectrophotometric curve Fig. 6, Chart 2.

This filter transmits a very narrow band having a maximum transmission at 340  $\mu$ . It is similar to filter No. 5 but with the transmission band moved slightly in the direction of longer wave-lengths. This displacement is obtained by increasing the concentration of copper sulfate solution, thus absorbing all wave-lengths shorter than 315  $\mu$ ; and by decreasing the concentration of the nickel sulfate thus moving the cut, caused by this material, somewhat in the direction of the long wave-lengths. Although this filter is almost ideal from the standpoint of narrowness and the consequent purity of radiation, its high absorption is a serious disadvantage in that it unduly decreases the intensity factor of the transmitted radiation. Unless measures are taken to increase the intensity of radiation incident on the filter or to prolong the exposure time, it may



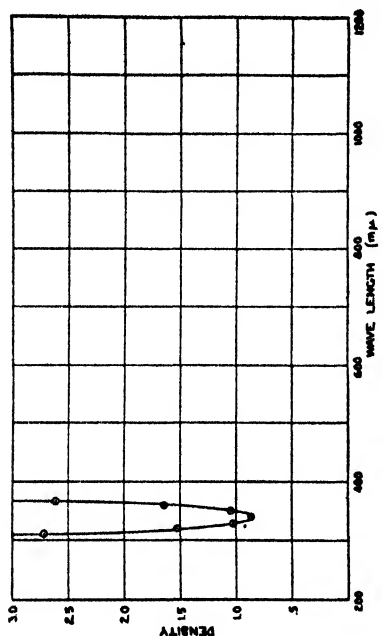


FIG 5

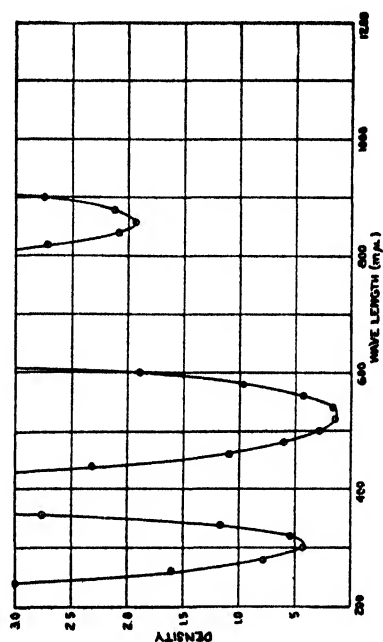


FIG 6

CHART 2. Spectrophotometric absorption curves for filters Nos. 5, 6, 7, 8.

FIG 7

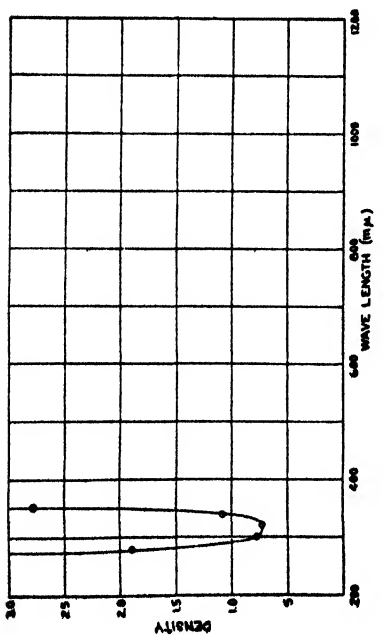


FIG 7

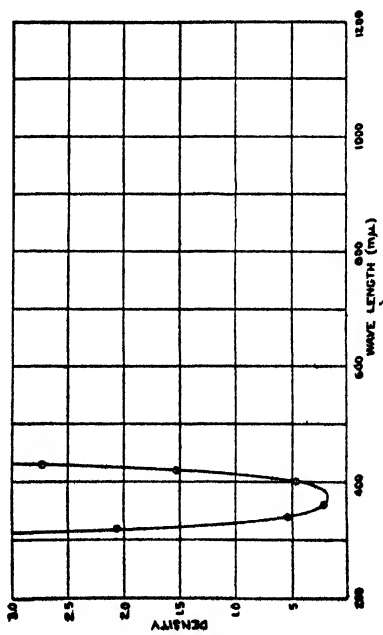


FIG 8

be difficult to determine definitely whether any apparent impotency of the radiation transmitted by this filter is due to its wave-length characteristics or to the low value of the radiation intensity transmitted.

*Filter No. 7—Ultra-violet*

Composition: Corning glass G586                      0.32 cm.  
Solution  $\text{CuSO}_4, 5\text{H}_2\text{O}$  (15.6%)      2.0 cm.

Absorption characteristics, spectrophotometric curve Fig. 7, Chart 2.

This filter transmits a rather broad band in the near ultra-violet. Its maximum transmission is high, being 70 per cent at  $368 \text{ m}\mu$ . It completely absorbs radiations of wave-length less than  $317 \text{ m}\mu$ . The cut on the short wave-length side of the band is determined by the copper sulfate component while that on the long wave-length side is determined by the Corning glass G586. Although the filter is very satisfactory from the standpoint of total transmission characteristics, the transmission band is somewhat broader than is desirable.

*Filter No 8—Ultra-violet*

Composition: Corning glass G980A                      0.32 cm.  
Solution  $\text{NiSO}_4, 7\text{H}_2\text{O}$  (50%)      2.0 cm.

Absorption characteristics, spectrophotometric curve Fig. 8, Chart 2.

This filter has a double transmission band, one having a maximum at  $304 \text{ m}\mu$  in the ultra-violet and the other at  $524 \text{ m}\mu$  in the mid-green of the visible spectrum. The residual transmission band having its maximum at  $830 \text{ m}\mu$  in the infra-red may be neglected since the maximum transmission in this region is only 12 per cent. It will be noted that the absorption of this filter is due entirely to the nickel sulfate solution, since the G980A glass is completely transparent between 200 and  $1200 \text{ m}\mu$ . The characteristics of the transmission band in the ultraviolet are excellent for the purposes for which these filters were designed. The maximum transmission, at  $340 \text{ m}\mu$ , is 40 per cent, while the limits extend from  $240 \text{ m}\mu$  to  $357 \text{ m}\mu$ . Thus far no absorbing material has been found which will eliminate the transmission in the green without seriously interfering with the transmission in the ultra-violet.

In some of the preliminary experiments Dr. Clausen found that very positive results were obtained by using filter No. 8. The question at once arose whether the effect was due entirely to the radiation in the ultra-violet, or was dependent to some extent upon that in the green. Filter No. 1, since it is practically identical with No. 8 in respect to its green transmission, was therefore used in later experiments to determine de-

finitely whether or not the presence of the green radiation transmitted by filter No. 8 along with the ultra-violet had any significance.

In Table I are summarized the characteristics of the eight filters described in this paper. In the column "wave-length limits" are shown the wave-length values at which the transmission of the filter is equal to 0.1 per cent ( $D=3.0$ ). It is considered that any transmission less than 0.1 per cent may be neglected and these wave-length values represent satisfactorily the "limits" of transmission. In the column "CG" are given the wave-length values which are estimated to represent the center of gravity of the transmission band. This in most cases is very nearly equivalent to the wave-length of the maximum transmission, but in case of asymmetrical transmission bands the two values may differ appreciably. In the last column of the table, "E," are the values of total energy transmitted by the various filters.

TABLE I  
SUMMARY OF CHARACTERISTICS OF FILTERS

No.	Color	Transmission		Wave-length Limits	CG	Residuals	E
		Maximum	%tr.				
1	Green	522	55	443-590	524	805 to 903 max. at 830 Tr. = 1.2%	0.042
2	Orange red	630	30	580-725	640	910 to 1300 max. at 1080 Tr. = 0.44%	.040
3	Infra-red	770	35	720-1120	860		.122
4	Blue	455	26	360-505	435	910 to 1300 max. at 1080 Tr. = 0.44%	.030
5	Ultra-violet	315	19	270-355	316		.005
6	Ultra-violet	340	14	315-370	340		.003
7	Near Ultra-violet	368	70	317-430	376		.012
8	Ultra-violet and green	304	40	240-357	305	804-903 max. at 830 Tr. = 1.2%	.113
		524	75	416-605	525		

The determinations of total energy transmissions were made under actual working conditions. These values were measured by means of a thermopile and sensitive galvanometer. The response of a thermopile depends upon thermo-electric forces generated by the absorbed energy

and is independent of the wave-length. Readings obtained are therefore directly proportional to total energy incident upon the thermopile. The thermopile was placed in the irradiation housing and occupied approximately the same horizontal plane as that occupied by the rats during the irradiation treatment. The light source was then operated under the same conditions as those used in the treatment of the animals. It is practically impossible to operate an arc lamp at constant intensity, since there are continual fluctuations in current which are due to several causes. It was necessary, therefore, to make several readings in order to obtain a satisfactory value. Working in this manner, we found it was impossible to obtain very good agreement between successive readings. A more elaborate arrangement was therefore made under laboratory conditions where the arc could be more precisely controlled than in practice. A set of filters, identical with those used in the irradiation work, was arranged and a second set of measurements made. The values given in Table I are the weighted mean of these two sets of observations. The absolute value of energy of course must be derived entirely from those measurements made under actual working conditions in the irradiation housing. The energy measurements made in the laboratory are somewhat more precise and reproducible and this series was given greater weight in determining the relative energy values of the various filters. The values given in Table I are in terms of calories per square centimeter per minute. It will be noted that filters Nos. 5 and 6 transmit relatively very little energy. The value for filter No. 7 also is relatively low. The energies transmitted by filters No. 3 and No. 8 are approximately equal. That transmitted by No. 1, when subtracted from that transmitted by No. 8, gives approximately the value of energy which is transmitted by the ultra-violet band of filter No. 8. A comparison of the green transmission bands in filters No. 8 and No. 1 will show that that of No. 1 is somewhat narrower and has a lower transmission maximum. It is probable, therefore, that in arriving at a value for the energy transmitted by the ultra-violet band of No. 8 a value somewhat greater than 0.042 should be used. It is estimated that the energy transmitted by the green and infra-red bands in filter No. 8 is approximately 0.050 calorie per sq. cm. per minute. This gives for the ultra-violet band of filter No. 8 a value of 0.63. This value is practically 50 per cent of that for filter No. 3.





## THE USE OF ISOLATED RADIATIONS IN EXPERIMENTS WITH THE RAT

### I. THE EFFECT OF INFRA-RED RADIATION ON THE GROWTH OF THE RACHITIC RAT

BY ETHEL M. LUCE CLAUSEN\*

*(From the Departments of Pediatrics; Yale University, New Haven, Conn.,  
The University of Rochester, Rochester N. Y., and Johns Hopkins  
University, Baltimore, Md.)*

Received for Publication—June 10, 1929

#### INTRODUCTION

OF RECENT years the study of radiation therapy has received a great impetus. The discovery of the value of ultra-violet radiation in the prevention and cure of rickets is so well known as to need little comment. Since this discovery, the use of radiation therapy has been extended to the treatment of many diseases and exaggerated claims have been made as to its value. Very little attention has been paid to the fact that full radiations from any source of light may include a wide range of rays extending from the ultra-violet, through the visible to the infra-red regions of the spectrum.

There is a tendency, in an ever-growing literature on the subject of light, to attribute every effect observed to radiations from the ultra-violet end of the spectrum. There is also a general impression that neither visible nor infra-red radiation exerts a specific effect on the animal organism. In order to know whether this is the case, it is necessary to isolate groups of radiations and to test their effect alone and in combination with other rays.

In 1922, Hess, Pappenheimer, and Weinstock (1) and Hess and Weinstock (2) realised the importance of working with light filters. In an attempt to isolate and define the limits of anti-rachitic radiations they used a series of filters and tested the effects of radiations transmitted by them on rats fed on a standard rickets-producing diet. They found that the anti-rachitic radiations were limited to a very narrow band, "between 296–302  $\mu$ ," and that waves longer than this were without effect.

The studies reported in this paper are the result of an attempt, extending over a period of two years, to work with isolated radiations, of known

\*Medical Travelling Fellow of the Rockefeller Foundation and Senior Sterling Fellow of Yale University.

energy value and spectral distribution, and to test these radiations on rats suffering from various dietary deficiencies.

The filters used in these experiments have been supplied to me by Dr. C. E. K. Mees to whom I am greatly indebted for a cooperation which has rendered this research possible, and also for much helpful criticism.

### METHOD

The method employed in these experiments has involved the use of:

1. A series of filters made of materials with a selective absorption, which transmit narrow bands of radiation of known spectral distribution, whose energy value is measured against the source of light used.

2. A source of light giving an approximately continuous spectrum.

3. An irradiation apparatus made to fit the filters.

4. A photographic dark room for the housing of animals.

1. *Filters:* The filters used in these experiments have been designed by Mr. L. A. Jones of the Eastman Kodak Company. A full description of them has been given in this journal (3). For a series of filters of general usefulness see also L. A. Jones (4).

2. *Source of Light:* The source of light used has been the same throughout all the experiments, viz. a "Solarite" carbon arc lamp burning "white flame" copper coated carbons. ("Therapeutic A," of the National Carbon Company, 12 inch  $\times$  13 mm.). The lamp was run on a direct current at 110 volts and 30 amperes and was enclosed in a brightly polished reflector as shown in Figure 1. The reflector was made of metal, plated inside with highly polished nickel to obtain a surface giving the maximum reflection of light. The lamp was suspended immediately above the irradiation apparatus as shown in Figure 1.

3. *Irradiation Apparatus:* Figure 1 is a diagram, drawn to scale, of the irradiation apparatus used. It consists of a strong wooden box supported on a table by three levelling screws. (Figure 1, diagram 1, gives a longitudinal, diagram 2 a transverse, view in section of the apparatus). The box is blackened inside and carries two metal ventilators above and below at the back, and one below in the front as shown in diagrams 1 and 2. The apparatus is open below and the ventilation thus obtained was found to be completely satisfactory. Inside the box are two narrow wooden projections (E and G diagram I); for holding an irradiation cage (G) used for rats, and a tray (E) used for the raying of diet. Filters A and B fit the apparatus as described below.

*Filter A* fits directly into the top of the wooden box. It consists of a metal tray 11 inches wide, 18 inches long and 2 inches deep, made of cast

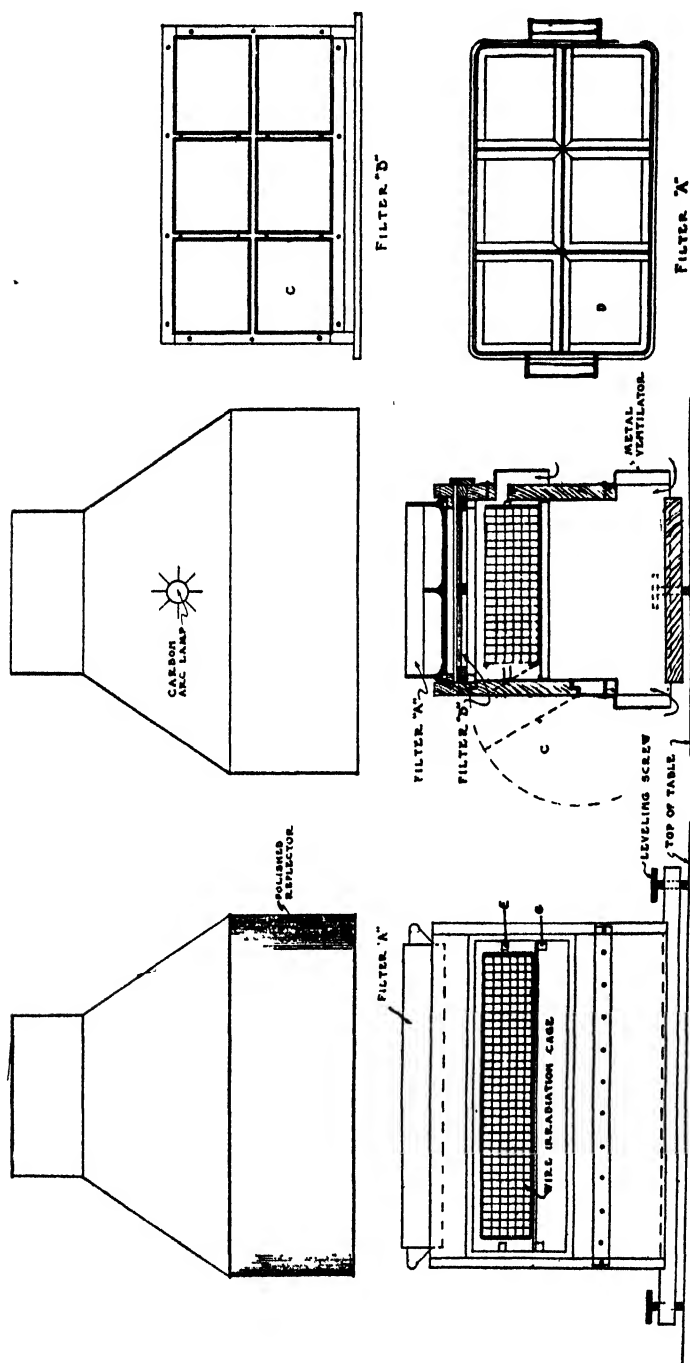


DIAGRAM 2.

DIAGRAM 1.

SCALE 0 1 2 3 4 5 6 7 8 9 10 11 12 INCHES

Figure 1. Irradiation apparatus.



brass heavily coated with silver. In the bottom of the tray are six rectangular openings (D)  $4\frac{1}{2}$  inches wide and  $5\frac{1}{2}$  inches long. Glass filters, protected at the edge with gasket material, can be screwed into these openings in such a way as to form a water-tight cell. This cell will hold solutions to a depth of 2 cm.

Jones (4) emphasises the necessity for using suitable gasket material in order to maintain a tight joint in these trays, during periods of irradiation. In preliminary trials we had trouble with leakage, but the gasket material recommended by him (pure unvulcanized gum rubber sheets) was found to be entirely satisfactory.

*Filter B* slides into the box from behind. It consists of a wooden holder containing six rectangular openings (C) the same size as those in Filter A which can be filled with gelatine filters. Filter B fits directly underneath Filter A as shown in diagram 2 and by this method the gelatine is protected from the direct action of the radiation. In cases when no gelatine filter is required, an unfilled Filter B is used so as to fill up the slit at the back of the apparatus and prevent the entrance of scattered radiation.

It is seen from this diagram that the radiations from the carbon arc lamp are thrown on to the irradiation apparatus by means of the reflector with a minimum loss of light. They pass first through the solution in Filter A, then through selectively absorbing glass, then if need be through dyed gelatine, before finally impinging on the rats in the irradiation cage. It is possible by the use of this apparatus (a) to obtain a concentration of light and reduce the scattered radiation to a minimum; (b) to ensure that the animals receive no radiation that has not been filtered in the manner desired.

**4. Photographic Dark Room:** The animals used in the experiments were housed in a room specially designed for photography, 17.4 ft. long; 16.4 ft. wide and 10.8 ft. high. It was shut off from the light by double doors each 3 ft. wide; the outer door opened into a laboratory which was used for the irradiation of the animals. The dark room was ventilated by means of an electric fan placed at the top of the inner door, on a level with an air shaft which opened into the wall opposite.

The room was kept completely dark except for a period of about 2 hours daily when the animals were fed and irradiated. During this time a 10 watt Westinghouse Mazda Lamp, enclosed in green frosted glass such as is used in photographic work, was used to give the necessary illumination.

**Heating of Room:** The room contained no radiators. It was heated by means of hot air pipes covered with an insulating material containing

80 per cent magnesia. The amount of heat from the pipes maintained a fairly constant temperature of 70° F in the room during the winter. In summer it rose higher, as discussed later.

The rats were kept in galvanised iron wire cages (8×10×10 inches) made with a  $\frac{1}{2}$  inch mesh, and had no bedding. Food and water were supplied in glass jars which were changed daily. Each animal was weighed once a week. The cages were supported on wire stands  $1\frac{1}{2}$  inches high with a zinc pan placed underneath to catch excreta. Faeces and urine fell through the meshes of the cage to the pan beneath, and the daily technique of keeping the animals clean simply consisted in replacing soiled by clean pans. The cages were kept on tables covered with oilcloth, which were washed daily when the pans were changed. This technique for the care of the animals was found to be both quick and efficient. Speed was necessary in view of the desirability of having the room illuminated (even dimly) for the minimum time each day.

#### DAILY ROUTINE OF IRRADIATION

The carbons of the lamp were first adjusted to ensure that they would burn for the required length of time. Filter A filled with the required glass was then fixed in position, and the necessary solution poured in to a depth of 2 cm by measurement; Filter B, (filled or unfilled with gelatine) was placed in position and the level of the solution in Filter A was tested in all the cells and adjusted if necessary.

The rats were then brought out from the dark room in a closed container (6 to 9 could be rayed at one operation) and put into the wire cage. A wooden door (C diagram 2) which fitted in front of the irradiation cage was immediately closed. The lamp was then turned on for the required length of time, then shut off, and the rats were transferred back to the dark room in the closed container.

#### RE-MEASUREMENT OF FILTERS

Fresh solutions were not made up daily, but were kept up to the required concentration by the addition of distilled water to compensate for evaporation. About 3 liters of the solution to be used were made up at the beginning of the experiment. Enough of this to give a level of 2 cm. in Filter A (Figure 1) was used. After use each day the solution was washed back from Filter A into the stock bottle. A sample of each solution used was remeasured after daily use during an experiment lasting for 10 weeks. No change had occurred in the absorption of any of the solutions during this time. This is evidence (a) of the stability of the ingredients used in

the different solutions; (b) that it is possible with care to keep the concentration constant by the addition of distilled water, and so to avoid the necessity of making up new solutions.

Spectrophotometric measurements were made of the glass filters at the beginning and end of experiments to determine whether any change of absorption had occurred with use. It has been found that certain filters which transmit ultra-violet radiation are unstable. These filters may become opaque to short wave-length radiation after prolonged exposure to light of high intensity. We found that the intensity used in our experiments was not sufficient to alter the transmitting power of the glass filters used. Samples measured before and after use showed identical absorption curves. The re-measurement of filters after use is, however, a necessary precaution.

#### CARE OF FILTERS AFTER USE

After use each day the solution in Filter A was washed out and the filter carefully dried and polished. The gelatine filter was also removed from the apparatus and kept free from dust. Care was also taken to keep the inside of the reflector well polished. These apparently trivial points are emphasised because of their extreme importance in experiments whose aim is the quantitative use of radiation.

#### EXPERIMENTAL RESULTS IN A PRELIMINARY EXPERIMENT ON THE EFFECT OF RAYING RACHITIC RATS THROUGH A SERIES OF FILTERS TRANSMITTING NARROW BANDS OF RADIATION

The rachitic rat was used in these experiments because of its known susceptibility to ultra-violet radiation.

In an orientation experiment we used seven filters, full details of which are given by Jones (3) (Filters 2, 3, 4, 5, 6, 7, 8).

Three families of rats, containing 8 members in each family (from the Albino Supply Co., Philadelphia) were used in this experiment. They were divided into 8 groups, each group of three rats containing one member of each family. All the rats were fed on Steenbock's<sup>8</sup> modification (5) of McCollum's 3143 diet. One group was kept all the time in complete darkness, the other seven groups were rayed daily for 10 minutes, each group of three rats receiving radiations through one of the seven filters. The experiment was continued for a period of 70 days. At the end of this time the rats were killed, and an ash analysis was made of the bones of both legs, (femur, tibia and fibula). The bones were dried for 12 hours at

110° C, extracted in hot alcohol for 48 hours, followed by 12 hours extraction in hot ether. The fat-free bones were then ashed in porcelain crucibles at red heat till all organic matter had disappeared.

*Result of Experiment:* The main result of this experiment is seen from Fig. 2 where the average ratios between the ash and organic residues (A/R values Chick and Roscoe (6)) in the bones of each group, are plotted against the wave-length at the centre of gravity of the band transmitted by each filter (C. G.). It is seen that the best protection, as indicated

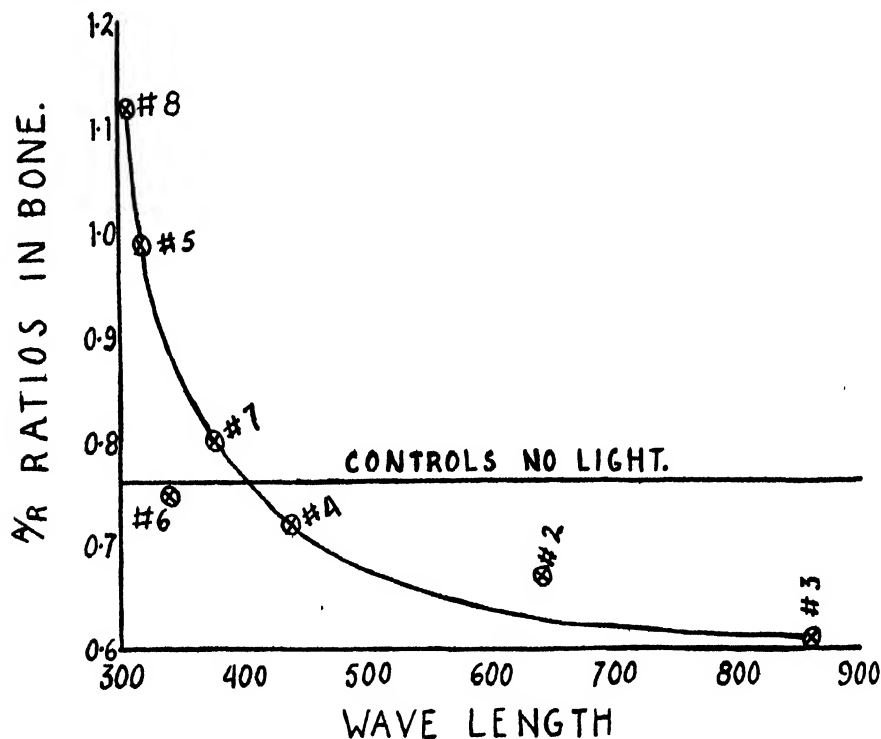


FIG. 2.—Average A/R values, 3 rats in each group, plotted against C.G. wavelength of filter used.

by the A/R ratio, was obtained with radiations transmitted by filter 8 which had a maximum transmission in the ultra-violet of 40 per cent at 304  $m\mu$  and a wave-length limit in the ultra-violet from 240–357  $m\mu$ . This filter also transmits a band of radiation in the green, which in a subsequent experiment was tested separately and found to be without effect.

This experiment illustrated some of the difficulties met with in attempts to work qualitatively and quantitatively with radiation.

1. In general, in attempting to obtain narrow spectral bands by selective

absorption methods, it is necessary to sacrifice intensity in order to obtain purity of ray. This will lead to inconclusive results such as those obtained with the use of filters 6 and 5. The results obtained with the use of both these filters can be interpreted to mean (a) That the rays transmitted were inactive; (b) That they were not transmitted in sufficient intensity to produce either a full (No. 5) or a partial (No. 6) result.

2. There is a great variability in the percentage of radiation transmitted at the wave-length of maximum transmission in each filter. The range in this series of filters is from 14–75 per cent. See Jones (3)

3. There is also of necessity great variability between the total energy value of the different groups of radiation. The two filters that transmit approximately the same total energy are 8 ( $0.113 \text{ gm cal/min/cm}^2$ ) and 3 ( $0.122 \text{ gm cal/min/cm}^2$ ). See Jones (3)

4. In attempting to analyse the data obtained from three rats in each group we found a wide range of variation within the groups themselves. Although the mean values could be expressed in the form of a smooth curve (Fig. 2), the probable error of the mean in each group was such that there was much over-lapping. We decided, therefore, to work in future experiments with large groups of animals so as to obtain results which were capable of statistical analysis.

#### EXPERIMENT II, WINTER 1928

The object of this experiment was to determine whether near infra-red radiation within the limits transmitted by filter 3 ( $720\text{--}1120 \text{ m}\mu$ ) exerted any effect on rachitic rats. The orientation experiment, already described, indicated an effect which resulted in lower A/R values in bone in a group of three rats receiving infra-red radiation daily for a period of 70 days. This result was not conclusive because of the limited number of animals used.

We decided to test the effect of infra-red radiation (a) given alone; (b) given in addition to ultra-violet. For this purpose we selected filters 3(infra-red) and 8 (ultra-violet). The energy value of the radiations transmitted through these two filters is approximately the same (i.e. for 3,  $0.122 \text{ gm cal/min/cm}^2$ ; for 8,  $0.113 \text{ gm cal/min/cm}^2$ ). Filter 8 transmits, in addition to ultra-violet, a band of radiation in the green. It has also a slight residual transmission in the infra-red. As indicated by Jones (3) Table I, the maximum transmission at  $830 \text{ m}\mu$  is only 1.2 per cent with a wave-length limit of  $805\text{--}903 \text{ m}\mu$ . This band is therefore very narrow; the total energy transmitted in this region must therefore be exceedingly small and, our experiments show, of negligible magnitude. We wished to find out wheth-

er the band in the green contributed to the result obtained with this filter. It was found impossible to exclude the green radiations from this filter without reducing the intensity of the ultra-violet, but the use of filter 1, Jones (3), made it possible to exclude the ultra-violet and use the green.

Full details of the radiations transmitted by these three filters are given by Jones (3) (Nos. 1, 3, 8).

Ninety rats (from the Albino Supply Co., Philadelphia) were used in this experiment; 9 litters containing 10 members in each litter. These rats were divided into five groups of 18 rats in each group, each group containing 2 members out of each family sampled by the method described below. All the rats were fed on Steenbock's modification (5) of McCollum's 3143 diet and radiated as follows:

### *Groups*

Controls Kept in complete darkness.

1 Rayed for 10 minutes daily through filter 1.  
Green radiations (443–590  $m\mu$ ).

3 Rayed for 10 minutes daily through filter 3.  
Infra-red radiations (720–1120  $m\mu$ ).

3+8 Rayed for 10 minutes daily through filter 3.  
Followed by a similar irradiation through filter 8 (ultra-violet and green)

3 720–1120  $m\mu$

8 240–357  $m\mu$

416–605  $m\mu$

8 Rayed for 10 minutes daily through filter 8.  
Ultra-violet and green.

240–357  $m\mu$

416–605  $m\mu$

### SAMPLING OF FAMILIES IN THE SELECTION OF GROUPS

Great care was exercised in the sampling of animals for the different groups, to secure both equal representation of the different families and equal average weights of the animals in each group. Diagram I represents the method employed.

The width of the wedge indicates gradation of weight from the highest to lowest in individual members of a family, and in the mean initial weights of the families. The groups were selected as indicated on diagram. The rats were 24 days old at the beginning of the experiment and the mean initial weight per rat, in the different groups, was as follows:

	gm.
Controls	35.1±0.56
1	35.0±0.54
3	34.2±0.75
3+8	34.9±0.63
8	34.9±0.70

This method of sampling rats is emphasized because it has been found effective in reducing errors due to individual variation of the rats in the same group.

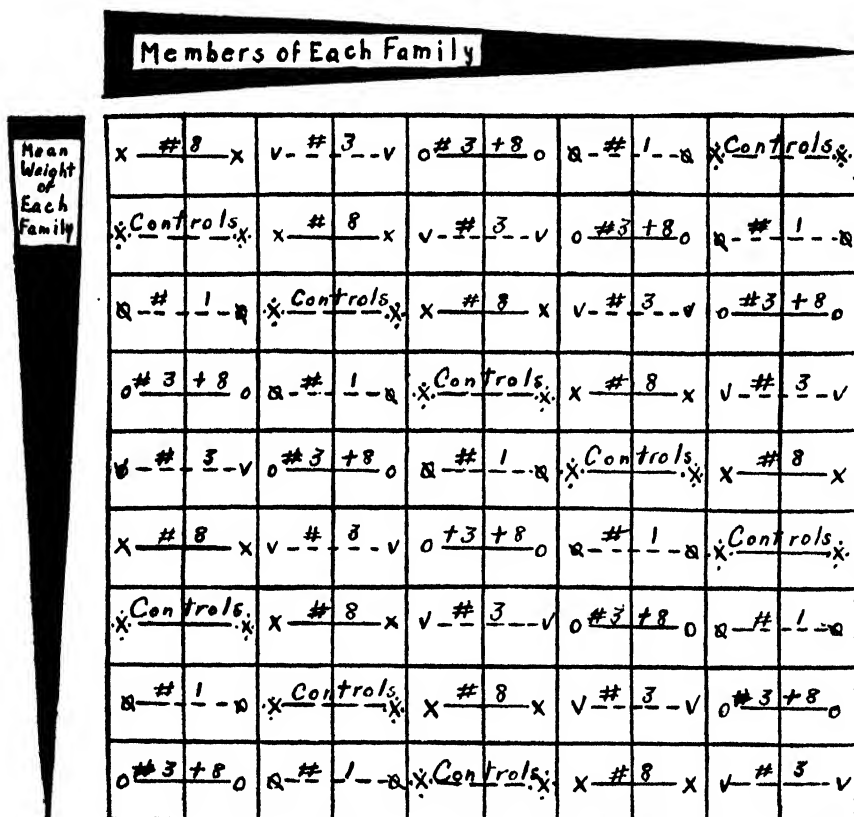


Diagram I

DIAGRAM I.—To illustrate method of sampling rats. Width of wedge indicates gradation of weight.

### COURSE OF EXPERIMENT

The experiment was continued for 49 days, and was then terminated because the control rats and the members of group 1 were moribund with

rickets. For the first four weeks of experiment nothing very striking was observed. The different groups of rats followed the usual course of protected and unprotected rats fed on a standard rickets-producing diet, i.e. the controls group 1, and group 3 grew less well than groups 3+8, and 8, and showed no clinical evidence of receiving protection against the disease. After the 35th day, however, a striking effect on growth was

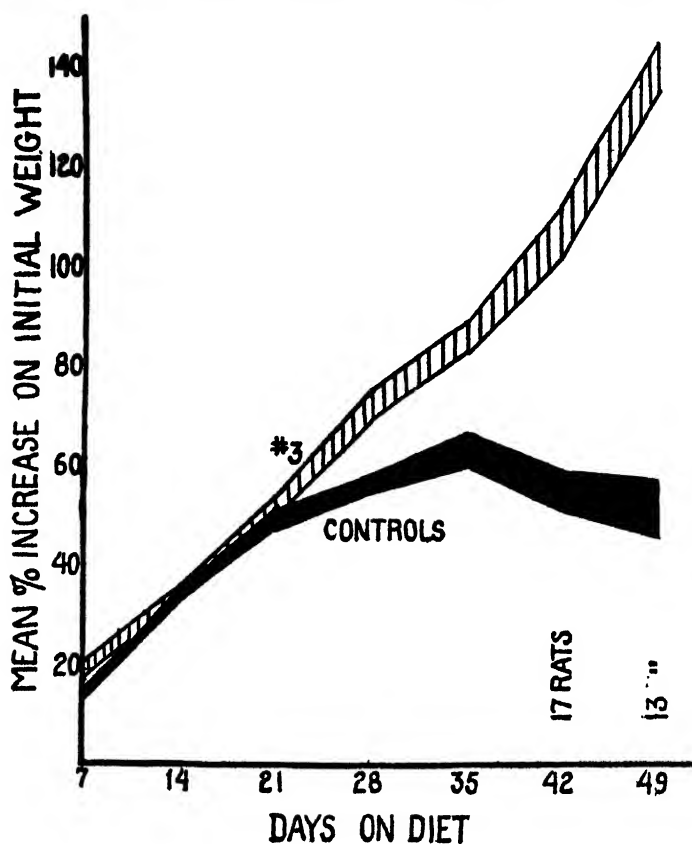


FIG. 3.—A comparison of the growth of two groups, 18 rats in each group.

Controls, no radiation.

No. 3 infra-red radiation.

Width of band—P.E. of mean.

observed in group 3 receiving infra-red radiation. At the stage in which the controls and group 1 began to lose weight, develop hind leg paralysis and all the terminal signs of extreme rickets, group 3 was in a different category. These 18 rats continued to grow, and ate about 5 grams a day more food than the controls, although clinically they showed no evidence of protection. Their chests were soft, rachitic deformities could be felt,



and there was obvious enlargement of the radioulnar epiphyses. None of them developed hind leg paralysis and they remained fairly active to the end of the experiment.

The clinical course of groups 3+8 and 8, was identical. The animals in both groups were active, showed steady growth, had firm, well developed

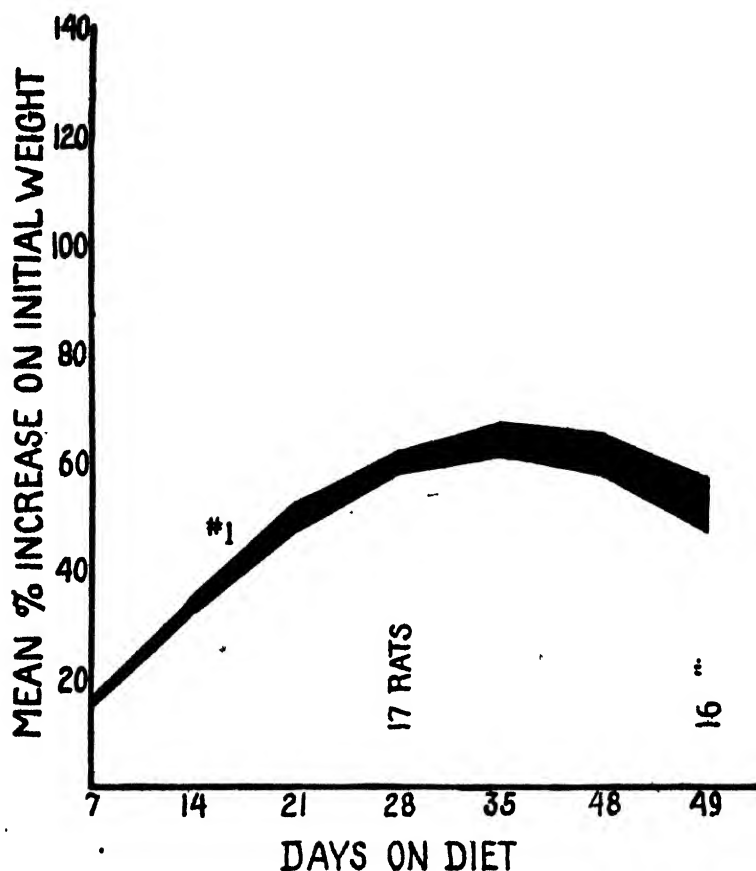


FIG. 4.—No. 1 green radiation.  
Width of band—P.E. of mean.

chest and no clinical evidence of rickets. Towards the end of the experiment, group 3+8 appeared to be growing slightly better than group 8, but the difference at the time was so slight that we regarded it as being without significance. Its significance will be discussed later. Figs. 3, 4, and 5 show the growth of the different groups. In these charts the mean percentage increase on initial weight in each group is plotted against the

length of time on the diet. The probable error of the mean for each group, is indicated by the width of the band.\*

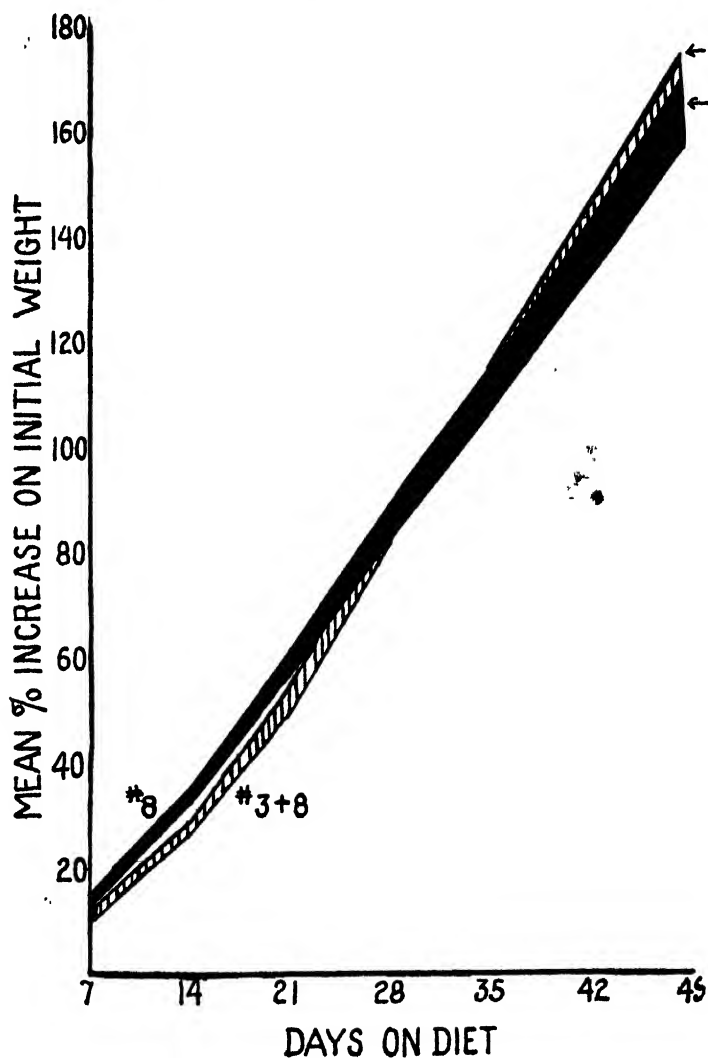


FIG. 5.—A comparison of the growth of two groups, 18 rats in each group.

No. 8 ultra-violet and green radiation.

No. 3+8 infra-red+Uv. and green.

Width of band—P.E. of mean.

\*The probable error of the mean in all the experiments has been calculated from the formula:

$$P. E. of Mean = .675 \sqrt{\Sigma(m-x)^2/n(n-1)}$$

$m$  = the mean

$x$  = any one of the observations

$n$  = the total number of observations

## POST MORTEM FINDINGS

At autopsy the rats were divided into three categories.

1. *Extreme rickets with hind leg paralysis*—controls and group 1. These 34 rats (one out of each group died early in the experiment) all showed the same well-known clinical picture—poor nourishment, chest deformities with multiple spontaneous fractures of the ribs, beading, enlarged epiphyses, marked lordosis of the spine.

2. *Extreme rickets without hind leg paralysis*—Group 3. By gross examination the degree of rickets in this group was just as severe as that in the control group and group 1, the only difference being that it occurred in a better nourished rat. The chests were deformed, there were multiple spontaneous fractures of the ribs, very marked beading and enlargement of the epiphyses, but no lordosis of the spine. Apparently the increased growth of these animals was sufficient, at this stage, to prevent spinal deformity followed by a mechanical involvement of the spinal cord. In a subsequent trial we found that with prolonged treatment the hind leg paralysis ultimately develops in rats receiving infra-red radiations.

3. *No Rickets*—Groups 3+8 and 8. These two groups were indistinguishable at autopsy. All the rats had firm, hard chests, normal epiphyses and showed evidence of complete protection.

## RESULTS OBTAINED FROM ASH ANALYSIS OF BONE IN THE FIVE GROUPS

A determination of the ash content in one leg bone (femur, tibia and fibula) from each rat was made by the method already described.

The results of these analyses are summarised in Table I. The data obtained from the A/R values have been expressed in the frequency polygons given in Fig. 6. It is seen from this (Fig. 6) that the control group, and group 1 are identical both on average values and distribution, so that the probable error of the mean is the same in both these two groups. This shows conclusively that the green radiations, transmitted through filter 1, are by themselves completely without effect. It does not show, however, whether the band of ultra-violet radiations would have been more, or less, effective without the green. So far we have been unsuccessful in isolating this band of ultra-violet radiations without reducing its intensity. It would be necessary to do this before we could say whether the band is more effective alone than mixed with the green.

This result also shows that the daily technique of handling the rats, and radiating them is completely without effect unless they are exposed to effective radiations.

TABLE I  
AVERAGE VALUES OBTAINED FROM BONE ANALYSIS. 18 RATS IN EACH GROUP

Rats rayed for 10 minutes daily through filters: 3+8 = Infra-red plus ultra-violet and green. 8 = Ultra-violet and green.

1 = Green Radiations. 3 = Infra-red radiations.

Group	Region of Spectrum	IN BONE									
		Dry Weight of Bone Grams	% Fat		% O.R.			% Ash			A/R
			Wet Wt.	Dry Wt.	Wet Wt.	Dry Wt.	Ext. Wt.	Wet Wt.	Dry Wt.	Ext. Wt.	
Control	No Light	0.1734	4.0	10.9	24.7	68.7	77.3	7.2	20.1	22.6	0.29±0.005
1	443-590m $\mu$	0.1859	3.9	10.0	26.4	70.1	77.8	7.5	19.9	22.1	0.29±0.005
3	720-1120m $\mu$	0.2271	6.2	14.9	22.3	57.2	66.5	11.1	28.6	33.1	0.49±0.012
3+8	(3) 720-1120m $\mu$ (8) 240-357m $\mu$ 416-605m $\mu$	0.3343	7.1	14.0	22.0	43.0	50.1	21.9	42.9	49.0	0.99±0.007
8	240-357m $\mu$ 16-605m $\mu$	0.3347	7.7	14.7	21.9	41.6	58.8	22.9	43.5	51.0	1.04±0.005

The frequency polygon of group 3 is wide-spread, diphasic, and quite different in character from that of the control group and group 1. The average value for the A/R ratios in this group is higher than in the other two unprotected groups. This apparent improvement in calcification cannot be regarded as evidence of protection. The average A/R values (0.49) are well within the limits obtained for unprotected rats, and furthermore, the clinical evidence of severe rickets was unequivocal. We

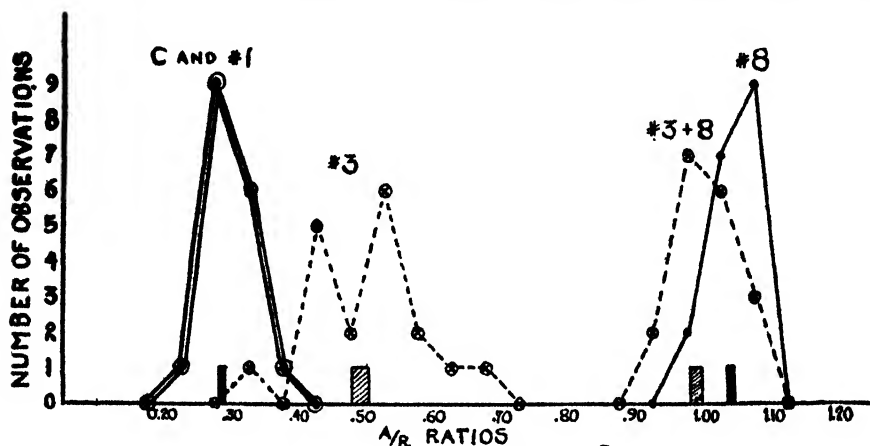


FIG. 6.—Frequency polygons of A/R ratios in bones of rats irradiated through filters.

C, controls, no radiation	17 rats	Small blocks under
No. 1, green radiation	17 "	curves denote place of
No. 3, infra-red radiation	18 "	mean with P.E. of mean
No. 3+8, infra-red + ultra-violet and green	18 "	in each group
No. 8, ultra-violet and green radiation	18 "	

attribute the higher A/R values in group 3 to an improvement in metabolic conditions following increased growth and food intake. We have shown in a subsequent experiment, prolonged for twice the time, that the improvement is only temporary.

The polygon of group 3+8 is more wide-spread than that of group 8, and the mean A/R values, including the probable error of the mean, falls below that obtained in group 8. We do not, on account of the limited number of observations, attach too much importance to the shape of these polygons, but it is interesting to note that the two groups showing the widest spread were these receiving infra-red radiation.

#### THE RELATIONSHIP OF THE RESULTS OF BONE ANALYSIS TO THE BODY WEIGHT OF THE RATS

The results obtained in group 3+8 as compared with group 8 were surprising. A comparison of Tables IV and V (pp. 151, 152) shows that

TABLE II  
AVERAGE VALUES OBTAINED FROM BONE ANALYSIS. SUMMER 1928

Group	Treatment	Number of Rats	Average Number of Days on Diet	Dry Weight of Bone Grams	IN BONE									A/R
					% O.R.			% Ash			% Fat			
					Wet Wt.	Dry Wt.	Ext. Wt.	Wet Wt.	Dry Wt.	Ext. Wt.	Wet Wt.	Dry Wt.	% Water	
Control	No Light	18	52	0.1533	23.1	63.4	76.6	7.0	19.2	23.4	6.3	17.2	63.5	0.31±0.007
3	Irradiated with Infra-red. 10 mins. daily	6 Increased growth	92	0.2039	25.2	72.3	78.3	7.0	20.2	22.7	2.7	7.7	65.1	0.28±0.006
3	Irradiated with Infra-red. 10 mins. daily.	12 No increased growth	46	0.1445	21.7	62.6	76.5	6.6	19.0	23.5	6.5	18.5	65.2	0.31±0.010

group 3+8 had consistently lower A/R values in 16 out of 18 rats as compared with their litter mates in group 8. The differences are small, but consistent, and the mean A/R value including the P. E. of the mean (Fig. 6) for the group 3+8 falls well below that of group 8. This result on the analysis of bone, was the first definite indication we had that infra-red radiation given in addition to ultra-violet produced any effect, and we were at a loss to explain it.

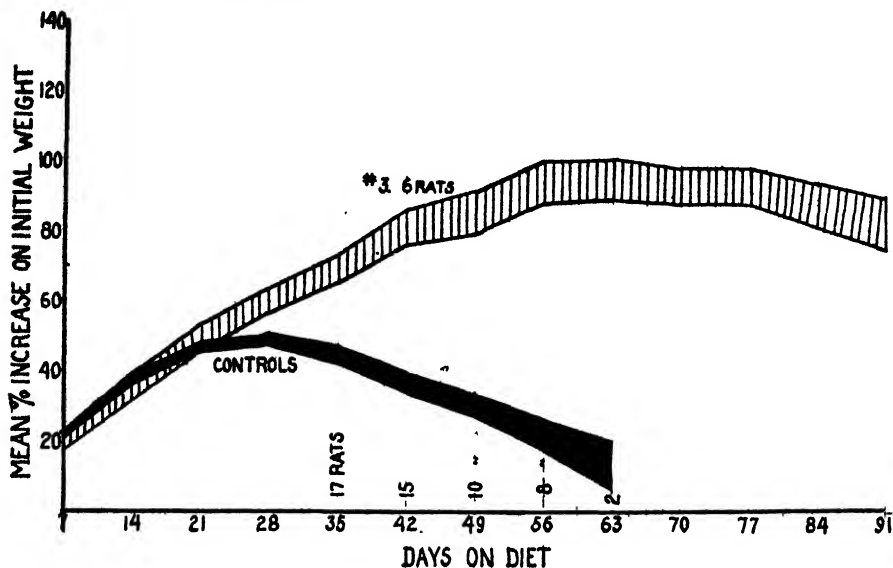


FIG. 7.—A comparison of the growth of two groups.  
 Controls, no radiation.  
 No. 3 infra-red radiation.  
 Width of band—P.E. of mean.

Since a lowered A/R ratio in one group must mean either an increase in the total organic residue in the bone of that group, or else a decrease in the ash, or both, we analysed the actual weights of ash and organic residue obtained from individual bone analysis of the 36 rats in these two groups. The average figures obtained were:

GROUP	O.R.	ASH
	gm.	gm.
3+8	0.1441	0.1437
8	0.1392	0.1446

These figures show that the group 3+8 had a definitely higher content of organic residue, and a slightly lower ash in bone than group 8.

We had observed a striking effect of infra-red radiation on the growth of the rats in group 3, and also a tendency to increased growth of the rats in group 3+8. We therefore analysed the data given in Chart 4 still further to find out whether the weight of ash and organic residues in the bones, bore any definite relationship to the body weight of the rats.

TABLE III

SHOWING THE RELATIONSHIP BETWEEN THE ACTUAL WEIGHT OF ORGANIC RESIDUE AND ASH IN BONE TO THE BODY WEIGHT OF RAT.

Average values obtained from 18 rats in each group.

Group	Average Body wt. Gm.	Average Organic Residue in bone. Gm.	Average Ash in bone Gm.	$\frac{\text{O.R.}}{\text{B.W.}}$	$\frac{\text{A}}{\text{B.W.}}$
Controls no light	52	$0.1187 \pm .002$	$0.0351 \pm .001$	$0.0024 \pm .00005$	$0.0006 \pm .00002$
1 Green	53	$0.1297 \pm .003$	$0.0371 \pm .003$	$0.0025 \pm .00005$	$0.0007 \pm .00002$
3 Infra-red	81	$0.1287 \pm .002$	$0.0644 \pm .002$	$0.0016 \pm .00002$	$0.0008 \pm .00002$
3+8 Infra-red + ultra-violet and green	94	$0.1441 \pm .003$	$0.1437 \pm .003$	$0.0015 \pm .00002$	$0.0015 \pm .00002$
8 Ultra-violet and green.	91	$0.1392 \pm .002$	$0.1446 \pm .003$	$0.0015 \pm .00002$	$0.0016 \pm .00002$

The results of this analysis are given in Table III and Fig. 8. Table III gives the average weights of organic residue and ash in each group and the ratios between these and the body weights of the rats. Fig. 8 gives the frequency polygon obtained from the analysis.

It is seen from Table III that the average values for organic residue in the bones of the five groups do not vary within very wide limits, the lowest value being 0.1187 gm. in the control group, and the highest 0.1441 gm. in group 3+8. As a result of this the OR/BW ratios (Fig. 8) reflect the *growth* of the rats so that the highest values are obtained in the two groups (controls and 1) with the lowest body weight. There is a much wider range of variation in the ash content of the different groups (0.0351 gm.



in the controls—0.1446 gm. in group 8), so that the ash content of the protected rats (8 and 3+8) is roughly four times as great as that in the other groups. The A/BW ratios therefore reflect the *degree of calcification* obtained in spite of the increased body weight of groups 3, 3+8, and 8. Group 3 therefore shows an OR/BW ratio which falls within the limits of the well-grown protected groups, whilst its A/BW ratio falls within the limits of the poorly calcified unprotected groups.

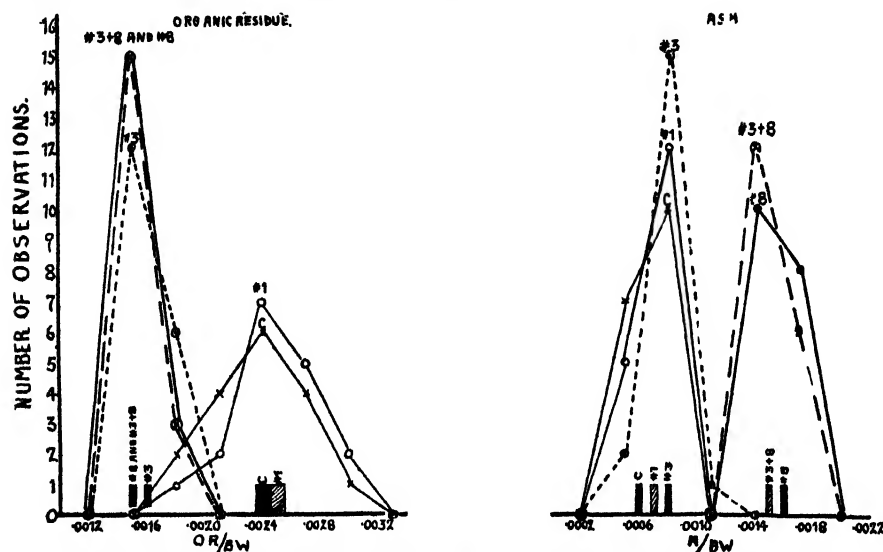


FIG. 8.—Frequency polygons of ratios between results of bone analysis and body weight of rats irradiated through filters.

C, controls. No radiation	17 rats	
No. 1, green	17 "	Blocks under curves mark
No. 3, infra-red	18 "	place of mean with P.E.
No. 3+8, infra-red + Uv. and green	18 "	of mean in each group.
No. 8, ultra-violet and green	18 "	

In Fig. 6 we have shown that the A/R ratios in the control group and group 1 are identical on mean values and distribution. The OR/BW and A/BW ratios for these two groups in Fig. 8 show slightly different distributions, but the mean values with the probable error of the mean in the two groups do not show any significant difference. The OR/BW values for group 3 are lower, and the A/BW ratios higher than these obtained in the control group and group 1, giving the higher A/R ratios shown on Chart 4.

Our main object in doing this analysis was to find out whether the lowered A/R ratios in group 3+8 as compared with group 8 could be traced to an increase in organic residue, or to a decrease in ash per unit

of body weight. Fig. 8 shows that the OR/BW ratios in groups 3+8 and 8 are identical, both on average values and distribution, so that the probable error of the mean in these two groups is the same. These two groups of rats therefore had the same amount of organic residue in bone per unit of body weight. The A/BW polygons for groups 3+8 and 8 are not quite the same. They fall within the same limits, but the shape of the polygons is different. Group 8 shows a skewed curve which may indicate a tendency for the ash values to be higher in this group than in group 3+8.

Owing to the limited number of observations, too much emphasis cannot be laid on this, but the difference is in favor of a slightly lowered ash content per unit of body weight in group 3+8.

#### CONCLUSIONS EXPERIMENT II—WINTER 1928

(1) Eighteen rats fed on a rickets-producing diet and irradiated daily for 10 minutes with near infra-red radiation (720–1120 $\mu$ ) for 49 days showed a marked acceleration of *growth* as compared with 18 litter mates kept in complete darkness.

(2) This increased growth in the rats receiving infra-red radiation was associated with slightly higher ratios between the ash and organic residue in the bones of these rats. There was no evidence of protection by clinical examination.

(3) A band of green radiations transmitted along with ultra-violet, through filter 8, had, by itself, no effect.

(4) Eighteen rats irradiated daily with both infra-red and ultra-violet radiations showed slight evidence of acceleration of growth at the end of 49 days as compared with 18 litter mates receiving ultra-violet only.

(5) This increased growth was associated with lower A/R values in bone in the group receiving infra-red in addition to ultra-violet, as compared with the group receiving ultra-violet radiation only.

(6) The two groups of rats receiving ultra-violet, with or without the addition of infra-red radiation, had the same amount of organic residue in bone per unit of body weight.

(7) The rats receiving infra-red radiation in addition to ultra-violet showed a slightly lower ash content of bone per unit of body weight, as compared with 18 litter mates receiving ultra-violet only.

(8) The main effect of a 10 minutes daily exposure to near infra-red radiation (720–1120  $\mu$  total radiation intensity 0.122 gm. cal/min/cm<sup>2</sup>) in both protected and unprotected rats appears to be a stimulation of growth.

## EXPERIMENT III—SUMMER 1928

In order to confirm the result obtained in promoting growth with infra-red radiation, the winter experiment of 1928 was repeated in the summer of 1928. Thirty-six rats were used, 18 kept as controls and 18 irradiated daily for 10 minutes through filter 3. The rats were 24 days old at the beginning of experiment.

mean wt. of control group =  $35.1 \pm 0.54$

      "      "      "      group 3 =  $35.5 \pm 0.63$

These rats were sampled from litters by the method described in the preceding experiment.

## COURSE OF EXPERIMENT

This experiment was complicated by the excessive heat of summer. A large number of the rats in both groups died at a time when it was found impossible to keep the temperature of the dark room below 80°F. The highest temperature recorded was 87°F. Twelve out of the 18 rats irradiated with infra-red radiation showed no response to the treatment. Their growth corresponded with that of the controls and 9 out of the 12 were dead at the end of 49 days, the remaining 3 died at the end of 56 days.

The remaining six rats in group 3 confirmed the results already described in Experiment II. This experiment, instead of being terminated at 49 days as in experiment II, was continued with the surviving rats for 91 days. Fig. 7 shows a comparison of growth of the two groups, the controls and the six rats in group 3.

A comparison of Figs. 3 and 7 shows:

(1) That all the animals in the summer experiment grew less well than they did in the winter experiment; a result that we attribute to the excessive heat of summer conditions. The difficulties of conducting experiments with rats in the height of summer in this country are well known. It has been our experience that the vitality of the animals becomes depressed to such an extent that they may fail to respond to any form of treatment.

(2) In both experiments there was no marked divergence in growth until after the 28th day.

(3) In Fig. 7 it is seen that the six rats in group 3 continued to grow until the 56th day, whilst the controls began to lose weight after the 28th day. The six rats in group 3 maintained their weight until the 77th day and only showed a slight loss of weight by the 91st day. At the end of this time five out of the six had developed hind leg paralysis and all the signs of extreme rickets. They were then killed, since it was

evident that no protection against rickets was being obtained. If the experiment had been continued it would have been possible to keep these six animals alive for some weeks longer.

#### POST MORTEM FINDINGS

The control group and the 12 rats out of group 3 which failed to respond to infra-red radiation showed an extreme degree of inanition with accompanying signs of rickets and hind leg paralysis. The six rats that survived the experiment for 91 days were better nourished, but five out of the six showed lordosis of the spine with accompanying hind leg paralysis, together with all the other signs of extreme rickets.

#### RESULTS OBTAINED FROM ASH ANALYSIS OF BONE

These are summarised in Table II.

The average A/R values for the controls and the 12 rats in group 3 which did not respond with increased growth are identical (Table II). The level of the A/R ratios in the six rats in group 3 which did respond with increased growth fall slightly below the values obtained for the controls. This shows clearly that the increased growth in the latter group was not accompanied by any protection against the disease. It also confirms our original finding in an orientation experiment, prolonged for 70 days.

#### CONCLUSIONS EXPERIMENT III—SUMMER 1928

1. The growth-promoting effect of infra-red radiation on rachitic rats was again demonstrated in 6 out of 18 rats.

2. It is thought that the excessive heat of summer conditions of experiment interfered with the full result which was obtained in a similar experiment conducted in the winter. The temperature of the room in which the rats were kept was 10°C higher in the summer than in the winter experiment.

3. The six rats which responded to irradiation with infra-red survived the diet for at least 4 weeks longer than the controls. They ultimately developed hind leg paralysis and all the signs of extreme rickets.

4. The A/R ratios in the bones of these six rats were slightly lower than those obtained from the controls.

#### DISCUSSION OF RESULTS

The literature on the subject of the biological effects of infra-red radiation is scanty and inconclusive.

Several investigators have claimed that the action of ultra-violet radiation may be modified or annulled by the simultaneous use of light of longer wave-length.

In 1922, Hess, Pappenheimer and Weinstock (2) put forward evidence that the anti-rachitic value of ultra-violet radiation was inhibited by the presence of visible and infra-red radiations. The basis of their evidence was a comparison between G86B, a Corning white glass filter, and G586A, a Corning blue glass filter, in the transmission of anti-rachitic radiations. By a comparison of spectrograms they regarded these two filters as transmitting ultra-violet radiation of the same wave-length and intensity. They found that a partial protective effect against rickets was produced in rats rayed behind the blue glass, whilst the radiations filtered through the white glass were without effect. Their argument, therefore, was that the same amount of ultra-violet radiation, whilst partially active alone, is inactive when mixed with light of longer wave-length. Azuma and Hill (7) criticised their results on the ground that they might equally well be explained in terms of unequal intensity of ultra-violet transmitted by the two filters. We know that different samples of the same glass may vary considerably in their transmission, and therefore it is impossible to say, without measuring the actual pieces used by Hess, *et al*, whether this is so. But, complete spectrophotometric measurement of samples (of the same thickness) of the two glasses made here\* shows that the white glass transmits slightly more ultra-violet, and at least seven times as much total energy in the infra-red, as the blue glass. This finding would lend support to the theory of Hess.

D. T. Harris (8) in 1925 found that the addition of visible radiations nullified the stimulating effect of ultra-violet on the metabolic rate of small animals, and on the smooth muscle of the frog. He regards this phenomenon as "one of physiological antagonism, rather than physical interference."

Azuma and Hill (7) repeated the experiments of Harris and were unable to confirm them.

Sheard and Hardenbergh (9) found that the simultaneous use of ultra-violet and infra-red radiation on demodex folliculum produced lethal effects more rapidly than the consecutive application of these two types of irradiation. This would imply that the two types of radiation were more effective mixed than separate. This view is directly opposed to

---

\* I am indebted to Mr. E. E. Richardson of Kodak Park for his kindness in making these measurements.

that of Pèch (10) who found that the effect of ultra-violet radiation in bleaching cotton, or in the production of erythema of the skin was delayed in its action on the addition of red, or infra-red radiation.

In 1918, Bovie and Klein (11) made the extremely interesting observation of a sensitizing action of ultra-violet upon the organism. They found that the exposure of paramoecia to a sub-lethal dose of ultra-violet rendered them so sensitive to heat that they could not stand a temperature which was optimal for the controls.

A recent paper by Sheard and Higgins (12) claims that the yellow and red wave-lengths of sunlight are important for the normal growth and development of chicks. These writers regard the presence of infra-red radiation as being without effect, but as they failed to exclude it from any of their filters, no conclusions as to its value or otherwise are permissible from their data.

Most of these observations are concerned with the reciprocal action of two types of radiation; none of them postulate any independent action of infra-red, or shows that by itself it will produce any physiological effect. This field is comparatively unexplored and the results reported in this paper do not, as yet, lend themselves to any but a tentative explanation.

We have demonstrated a growth-promoting effect on rachitic rats of an isolated band of radiations in the near infra-red region of the spectrum. The effect of infra-red radiation on these animals is different from that of ultra-violet in that it confers no protection against the disease.

Given in addition to ultra-violet, infra-red radiation also produces a slight effect on the growth of rats, as compared with those receiving ultra-violet only. At the same time the rats receiving infra-red radiation show an increase in the organic constituents of bone, a slight decrease in the ash, and lowered A/R ratios, as compared with those receiving ultra-violet only. This increase in organic residue is more apparent than real since it bears a definite relationship to body weight and apparently indicates only a slight increase in growth. The ash content of the bones of the rats receiving infra-red in addition to ultra-violet radiation is slightly lower per unit of body weight, than that in the bones of the group receiving ultra-violet radiation only.

The explanation of this is, at present, a matter of conjecture. We have considered various possibilities, amongst them that infra-red radiation modifies the synthesis of Vitamin D in the skin, under the action of ultra-violet, in such a way that its full effect is not apparent. This might be brought about (a) by a direct effect of infra-red radiation upon

Vitamin D already formed, or (b) by some independent action of infra-red radiation whereby the sterols in the skin are rendered less easily activated by ultra-violet.

We found in group 3 a marked increase, as compared with the other four groups, in the total fat of the skins of the rats. This increase in fat was not accompanied by any corresponding increase in the phospholipid or unsaponifiable fractions. We did not however, find this increase in fat in the skins of the rats in group 3+8.

I am indebted to Dr. Bloor, who kindly analysed samples of the fat extracted from the skins, for permission to publish the above statement. We can draw no conclusions from our data at present.

Sonne (13) found that 1.79 gm. cal/min/cm<sup>2</sup> of near infra-red radiation transmitted from a carbon arc lamp could be borne apparently indefinitely on the flexor surface of the human arm without giving rise to a blister. He also found it possible to raise the temperature in the blood underneath the skin several degrees by means of visible radiations, but not with infra-red. He concluded that infra-red rays are immediately absorbed in the superficial cells of the skin which are thereby quickly heated, evoking a sensation of pain at a comparatively low energy value of radiation; and because of this more heat is absorbed from visible than infra-red radiations. His work would indicate that the effect of infra-red radiation takes place at the surface of the human skin.

With regard to the possibility of the effects we have shown being due to *heat*, we do not claim to have ruled this out and we intend to investigate it further. It seems unlikely that the small amount of total energy used 0.122 gm. cal/min/cm<sup>2</sup> for 10 min. per day would be sufficient to heat the animals to any great extent. It was not sufficient to raise their rectal temperatures.

We have found that rats used in our experiments are very sensitive to heat; either to increased air temperature in summer or to an overdose of heat radiation. But the effects produced on the animals in these two ways are completely different from those obtained by radiation through filter 3. When overheated, the rats show evidence of exhaustion. They become inactive, lose appetite, fail to grow, show excessive sweating which starts around the chin and neck, and if heating is continued they die. The rats irradiated through filter 3 showed none of these symptoms. They were extremely active in the irradiation cage, and showed not the slightest evidence of sweating or exhaustion. We have also shown that 12 out of 18 rats in a summer experiment, when the air temperature was 10° higher than that in the winter, failed to respond to the radiation with

TABLE IV  
RESULTS OF BONE ANALYSES. GROUP 3+8

RAT		Rayed Through Filter	Region of Spectrum	IN BONE										
Serial Number	Family			Dry Weight of Bone. Gms.	% Fat		% O.R.		% Ash		% Water	A/R		
					Wet Wt.	Dry Wt.	Wet Wt.	Dry Wt.	Wet. Wt.	Dry. Wt.			Ext. Wt.	
55	A	3+8	(3) 720-1120m $\mu$ (8) 240-357m $\mu$ 416-605m $\mu$	0.3355	10.2	19.9	21.0	40.9	51.1	20.1	39.1	48.9	48.5	0.95
56	A	"	"	0.3680	6.4	12.9	21.9	44.3	50.9	21.1	42.7	49.0	50.6	0.95
57	B	"	"	0.3507	5.0	10.1	22.2	45.0	50.1	22.2	44.9	49.9	50.6	0.99
58	B	"	"	0.3443	2.9	5.9	23.1	46.5	49.5	23.5	47.5	50.5	50.5	1.02
59	C	"	"	0.3906	7.4	14.6	22.0	43.4	50.9	21.3	41.9	49.1	49.3	0.96
60	C	"	"	0.3777	8.1	15.3	22.1	42.0	49.6	22.5	42.7	50.4	47.3	1.01
61	D	"	"	0.3322	6.3	12.9	22.3	45.3	52.0	20.5	41.7	47.9	50.7	0.92
62	D	"	"	0.3650	5.1	9.9	22.1	43.4	48.3	23.7	46.5	51.7	49.1	1.07
63	E	"	"	0.3365	9.1	17.2	21.4	40.3	48.7	22.4	42.4	51.2	47.0	1.05
64	E	"	"	0.3300	8.4	16.4	21.5	42.1	50.5	21.1	41.4	49.5	48.8	0.98
65	F	"	"	0.3065	7.1	13.9	22.8	43.1	50.1	22.0	42.9	49.7	48.6	0.98
66	F	"	"	0.2860	5.9	12.0	22.4	45.2	51.3	21.2	42.8	48.6	50.3	0.95
67	G	"	"	0.3255	7.9	15.1	21.8	41.6	49.0	22.7	43.2	51.0	47.9	1.04
68	G	"	"	0.3575	6.3	12.1	22.7	43.7	49.7	22.9	44.1	50.2	49.4	1.01
69	H	"	"	0.2670	9.6	19.4	20.8	42.0	52.1	19.1	38.6	47.8	50.4	0.92
70	H	"	"	0.3185	8.4	16.4	21.3	41.4	49.7	21.3	41.8	50.2	49.2	1.01
71	I	"	"	0.3120	7.1	13.3	22.5	42.8	49.4	22.9	43.7	50.4	47.5	1.02
72	I	"	"	0.3149	7.4	14.1	22.1	41.7	48.4	23.5	44.4	51.6	46.9	1.06
			AVERAGE	0.3343	7.1	14.0	22.0	43.0	50.1	21.9	42.9	49.9	49.0	0.99 $\pm$ 0.007



TABLE V  
RESULTS OF BONE ANALYSES, GROUP 8.

Serial Number	Rar	Rayed Through Filter	Region of Spectrum	In Bone									
				Dry Weight of Bone, Gms.	% Fat		% O.R.			% Ash		% Water	A/R
					Wet Wt.	Dry Wt.	Wet Wt.	Dry Wt.	Ext. Wt.	Wet Wt.	Dry Wt.		
73	A	8	240-357m $\mu$ 416-605m $\mu$	0.3780	6.8	13.2	22.0	42.7	49.4	22.6	43.9	50.7	1.03
74	A	"	"	0.3515	8.8	17.2	21.3	41.6	50.1	21.1	41.2	49.8	0.99
75	B	"	"	0.2782	5.4	10.7	22.0	43.5	48.4	23.5	46.0	51.5	1.07
76	B	"	"	0.3223	5.8	11.2	22.2	42.9	48.4	23.7	45.8	51.5	1.07
77	C	"	"	0.3290	5.5	11.3	21.1	42.5	49.6	21.4	43.2	50.3	1.01
78	C	"	"	0.3148	6.4	12.5	21.6	42.8	48.2	23.2	45.3	51.7	1.07
79	D	"	"	0.3212	10.7	19.7	21.2	39.1	48.7	22.3	41.1	51.2	1.05
80	D	"	"	0.4423	7.2	13.6	21.9	41.3	47.8	24.0	45.1	52.2	1.09
81	E	"	"	0.3621	9.2	16.2	22.7	40.1	47.9	24.7	43.6	52.1	1.08
82	E	"	"	0.3637	8.9	16.8	21.1	40.2	48.3	22.9	42.9	51.6	1.06
83	F	"	"	0.3185	7.6	14.2	22.7	42.3	49.4	23.2	44.7	50.2	1.02
84	F	"	"	0.3137	11.5	21.7	20.3	38.5	49.2	21.0	39.8	50.4	1.03
85	G	"	"	0.3201	7.5	14.2	22.9	43.1	50.1	22.7	42.6	49.7	0.99
86	G	"	"	0.3322	6.4	12.8	21.6	43.0	49.3	22.1	44.1	50.6	1.03
87	H	"	"	0.3130	7.5	13.9	22.1	41.1	47.7	24.2	44.9	52.2	1.09
88	H	"	"	0.2967	7.8	15.0	21.7	41.5	48.8	22.8	43.5	51.1	1.04
89	I	"	"	0.3583	8.6	15.4	23.1	40.9	48.1	24.6	43.6	51.5	1.06
90	I	"	"	0.3090	8.1	15.3	22.5	42.4	50.1	22.4	42.1	49.7	1.00
			AVERAGE	0.3347	7.7	14.7	21.9	41.6	48.8	22.9	43.5	51.0	1.04 $\pm$ 0.005

infra-red. An increase in air temperature would therefore appear to depress the vitality of the rats to such an extent that they may be rendered unable to respond to treatment.

#### SUMMARY

1. A 10 minutes daily exposure to near infra-red radiation (720–1120  $m\mu$ ) total energy value (0.122 gm. cal/min/cm<sup>2</sup>) from a carbon arc lamp, will stimulate growth in rachitic rats and prolong their period of survival on the rickets-producing diet.

2. The effect of infra-red radiation on the rats is different from that of ultra-violet since the animals receive no protection against the disease.

3. An increase in air temperature during summer conditions of experiment will depress the vitality of the rats, and make them less likely to respond to treatment with infra-red radiation.

4. Given in conjunction with ultra-violet, infra-red radiation also stimulates growth, to some extent, in rats fed on a standard rickets-producing diet.

In conclusion my warm thanks are due to Dr. E. A. Park for his constant support and helpful criticism throughout the course of these experiments; also to my assistant Miss Ruth Teasdale for skilled and conscientious help in tedious routine work. I should like also to thank the anonymous donor of the Gift Fund of Yale University whose generosity has defrayed the cost of this research. My thanks also are due to Dean G. H. Whipple for the hospitality of the laboratories in the Medical School of the University of Rochester, where these experiments have been carried out.

#### BIBLIOGRAPHY

1. Hess, A. F., Pappenheimer, A. M. and Weinstock, M., 1922, *Proc. Soc. Exp. Biol. & Med.* XX, 14.
2. Hess, A. F. and Weinstock, M., 1923, *Jour. Amer. Med. Assn.* LXXX, 687.
3. Jones, L. A., *This Journal.* 1929, II, 1.
4. Jones, L. A., 1928, *Jour. Opt. Soc. Amer. and Rev. Sci. Instr.* XVI, 259.
5. Steenbock, H. and Black, A., 1925, *Jour. Biol. Chem.* LXIV, 263.
6. Chick, H. and Roscoe, M. H., 1926, *Biochem. Jour.* XX, 137.
7. Azuma, Y. and Hill, L., 1926, *Proc. Roy. Soc. B.* XCIX, 221.
8. Harris, D. T., 1925, *Proc. Roy. Soc. B.* XCVIII, 171.
9. Sheard, C. and Hardenbergh, J. G., 1927, *Jour. Parasitology*, XIV, 36.
10. Pech, J. L., 1920, *Comptes Rendues de l'Acad.*, 170<sup>a</sup>, 1246.
11. Bovie, W. T. and Klein, A., 1918, *Jour. Ger. Physiol.*, I, 331.
12. Sheard, C. and Higgins, C. M., 1928, *Amer. Jour. Physiol.* LXXXV, 290.
13. Sonne, C., 1921, *Acta. Med. Scandinavica*, LIV, 336.





## DO BAKING POWDER RESIDUES EXERT INJURIOUS EFFECTS UPON GROWTH AND NUTRITION?

BY WILLIAM C. ROSE AND FLORENCE L. CATHERWOOD\*

*(From the Laboratory of Physiological Chemistry,  
University of Illinois, Urbana.)*

Received for Publication—June 22, 1929

FROM time to time attention is directed in the literature to the possibility of injurious effects resulting from the habitual use in the diet of certain types of baking powders. This is particularly true of those powders which contain tartrates or aluminum compounds as the leavening agents. Investigations in many laboratories have shown that tartrates, when administered parenterally to animals, produce a severe form of nephritis associated with profound destruction of the tubular epithelium. Several years ago studies in this laboratory (Rose, 1924) demonstrated that the subcutaneous administration to rabbits of sodium tartrate leads to enormous increases in non-protein nitrogen, urea, creatinine, sugar and cholesterol of the blood, accompanied by diminished excretion of total nitrogen in the urine. In many of the animals the ability to excrete phenolsulfonephthalein dropped to zero. Pearce and Ringer (1913) report that the characteristic renal lesions occur regardless of the mode of administration of the tartrate, *provided* the latter reaches the circulation. On the other hand, it has been clearly shown that when tartrates are given by mouth, much larger doses are required than when they are introduced parenterally (cf. Underhill, Wells and Goldschmidt, 1913; Pearce and Ringer, 1913; Salant and Smith, 1914). In human subjects, Post (1914) was unable to obtain evidence of albuminuria or cast formation after giving medicinal doses of Rochelle salts to normal individuals, nor could he demonstrate an aggravation of an existing nephritis following oral tartrate administration. Thus there appears to be no doubt as to the nephrotoxicity of tartrates injected subcutaneously; but the evidence regarding the influence of these materials when administered orally is not so clear. Furthermore, it does not follow necessarily, because a single large dose by mouth does or does not lead to renal injury, that comparable effects would result from the alimentary introduction of small amounts over long periods of time.

\* Some of the earlier experiments reported herein were conducted by A. L. Rawlins.

As to the physiological action of aluminum-containing baking powders, Gies and his associates (1916) have maintained for a number of years that compounds of aluminum are absorbed from the intestine and may lead to deleterious effects. Findings adverse to the above, however, have been reported by a number of investigators. Thus the Referee Board of Consulting Scientific Experts (1914) found no detrimental influence upon metabolism following the ingestion of bread prepared with such baking powder. The experiments were conducted upon 26 healthy young men. Similar results were obtained by Schmidt and Hoagland (1919). Indeed, the latter state that aluminum is not absorbed from the intestine, but is quantitatively excreted in the feces.

Quite recently McCollum, Rask and Becker (1928) have applied the spectrographic technic to a study of the fate of administered aluminum. Although the method permits the detection of 0.5 parts per million of the metal, no evidence of the absorption of the latter from the alimentary tract was secured. The authors state that "Aluminum is not a constituent of either plant or animal matter," and that "Aluminum compounds in the diet in concentrations as high as 600 parts per million of the element aluminum exert no noticeable deleterious action on growth, reproduction, or general well being as judged by external appearance and autopsy." Similar conclusions regarding the non-toxic nature of aluminum were reached by Myers and his associates (1928). These authors observed no inhibition in growth nor other evidences of abnormality following the daily administration to rats of 2 mg. of aluminum in the form of potassium aluminum sulfate. Their results differ, however, from those of McCollum, Rask and Becker in regard to the occurrence of aluminum in biological materials. By means of a colorimetric method developed by Myers, Mull and Morrison (1928) minute traces of the element were detected in tissues of rats and dogs, and in human autopsy material.

In view of the lack of uniformity in the data briefly outlined above, and the apparent uncertainty which still exists in the minds of many as to the possible injurious effects of baking powder components, we have undertaken a series of investigations involving the use of three types of powders containing respectively (a) a tartrate, (b) calcium acid phosphate, and (c) a mixture of calcium acid phosphate and anhydrous sodium aluminum sulfate. In the present paper we are concerned only with the alleged injurious effects, especially the influence of the baking powders upon growth and renal function. In a later communication we shall consider the distribution of aluminum in animal tissues, and attempt to demonstrate whether it is an accidental or essential dietary component.

## EXPERIMENTAL

Young rats served as the experimental animals. These were divided into four groups, and each group was placed upon one of the diets outlined in Table I. As far as practicable, the animals of each litter were distributed among the four groups so as to make possible a comparison of the growth performance of each rat with that of its twin brothers and sisters upon the other rations. Inasmuch as the literature appeared to indicate a greater probability of nephrotoxic effects resulting from tartrates than from the other baking powder ingredients, a larger number of rats was placed upon Diet D than upon Diets A, B and C.

TABLE I  
COMPOSITION OF THE DIETS

	Diet A.	Diet B.	Diet C.	Diet D.
Casein, gm.....	80	80.0	80	80
Whole wheat flour, gm.....	760	760.0	760	760
Lard, gm.....	50	50.0	50	50
Cod liver oil, gm. ....	30	30.0	30	30
Salt mixture, gm. ....	40	40.0	40	40
Yeast, gm.....	20	20.0	20	20
Agar, gm.....	20	20.0	20	20
Milk, cc.....	530	530.0	530	530
Water, cc.....	70	70.0	70	70
Calcium phosphate baking powder, gm.....		64.5		
Calcium phosphate-sodium aluminum sulfate baking powder, gm.....			29	
Potassium bitartrate baking powder, gm.....				46

As will be observed, the diets are identical except for the baking powders which they contained. Diet A was devoid of all baking powder, and served as a control ration. The quantities of the powders employed were made to conform as nearly as possible with the directions on the containers. Inasmuch as the manufacturer's instructions are given in terms of teaspoonfuls per quart of flour, the corresponding weight proportions were determined by weighing several teaspoonfuls of each powder and several quarts of the mixed dry components of the diets. We have regarded the sum of the dry components as equivalent to the flour in ordinary bread. Inasmuch as the casein, yeast, salts, and agar, which were incorporated in our diets in order to make the food complete from the nutritive standpoint, reduced the proportion of flour in the mixture, it seemed fair to base the baking powder additions upon the weight of all ingredients exclusive of the lard and cod liver oil. On the other hand, the differences in

the proportions of the three powders employed in Diets B, C and D, are due to differences in the directions printed on the can labels.

In preparing each food the dry components, including the baking powder if present, were thoroughly mixed by hand, the lard and cod liver oil were then worked in, and finally the milk and water were added. The whole was made into a stiff dough, rolled into thin layers, cut into squares, and baked in an oven for 20 min. at a temperature of approximately 230° C. The final product was a light brown, crisp material, which was devoured greedily by the animals. From the loss in weight of the bread during baking, the food consumption of the rats, and the composition of the baking powder, it was possible to calculate the average daily intake of the leavening agents. These values will be discussed later. In addition to the bread, which was furnished *ad libitum*, each rat received 20 gm. of head lettuce at intervals of four days.

After 220 days upon the experimental rations, certain of the females upon each diet were bred with males upon the same ration. Of the young so obtained, 16 were placed at weaning upon diets identical with those of their parents with the exception that the quantities of baking powders per unit of mixed foods were doubled. Thus the second generation animals were subjected to the influences of the powders from the moment of conception until the end of the experiments. At the termination of the feeding periods the animals of both generations were anesthetized, killed by bleeding, and the blood samples subjected to analysis for non-protein nitrogen according to the procedure of Folin and Wu (1919). The kidneys were removed, weighed, and preserved for histological examination.

Seventy-two rats were employed in the investigation. In Tables II and III are shown for each animal the duration of the feeding period, the average daily intake of food and leavening agent, the final weights of the kidneys, and the non-protein nitrogen of the blood. As will be observed, the figures representing average daily food consumption of the animals upon the four diets are quite uniform for each sex. The values are all rather high, but this is to be accounted for by the fact that the food had a relatively low fat content and contained considerable moisture. A comparison of the weights of the kidneys of the animals upon the baking powder diets with those upon the control ration indicates that the leavening agents induced no hypertrophy. Indeed, the largest kidneys for each sex were found among the control animals. Rat 767♂ showed slightly enlarged kidneys, but the non-protein nitrogen of the blood was within the normal range for the species (45 to 50 mgm. per 100 cc). Rat 744♀ showed moderate hypertrophy with a very marked increase in the non-

TABLE II  
FIRST GENERATION OF RATS

Rat no. and sex.	Diet no.	Duration of experiment	Average daily food con- sumption	Average daily intake of			Final weight of kidneys		N.P.N. of blood
				P <sub>2</sub> O <sub>5</sub>	Al	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	Right	Left	
		days	gm.	mgm.	mgm.	mgm.	gm.	gm.	mgm. per 100 cc.
756♂	A	250	15.9				1.0	1.1	45.1
763♂	A	250	16.7				1.1	1.2	44.4
767♂	A	245	16.1				1.3	1.4	47.6
		Average	16.2				1.1	1.2	45.7
728♀	A	292	12.2				0.5	0.7	45.0
733♀	A	297	12.5				0.7	0.7	44.0
744♀	A	297	13.0				1.2	1.2	101.4
749♀	A	304	13.9				0.8	0.8	44.4
774♀	A	304	12.8				0.8	0.8	52.2
781♀	A	292	14.5				0.8	0.8	47.4
		Average	13.2				0.7	0.8	46.6
738♂	B	246	15.0	182			1.0	1.0	46.4
739♂	B	250	15.3	186			1.1	1.1	46.6
753♂	B	297	16.2	197			1.1	1.1	43.4
764♂	B	288	17.3	210			1.1	1.1	45.9
772♂	B	248	14.8	180			1.1	1.2	45.8
777♂	B	248	13.5	164			0.9	0.9	45.7
		Average	15.4	187			1.1	1.1	45.6
734♀	B	299	12.8	156			0.7	0.7	44.3
735♀	B	299	12.1	147			0.7	0.7	43.3
765♀	B	307	13.8	168			0.9	0.9	46.3
		Average	12.9	157			0.7	0.7	44.6
741♂	C	250	15.0	38	10.3		1.0	1.0	45.9
754♂	C	285	14.5	36	10.0		1.0	1.0	44.7
758♂	C	250	15.5	39	10.7		1.1	1.1	43.0
779♂	C	285	15.4	38	10.6		1.1	1.2	45.1
		Average	15.1	38	10.4		1.1	1.1	44.7



TABLE II (Continued). FIRST GENERATION OF RATS

Rat no. and sex.	Diet no.	Duration of experiment	Average daily food consumption	Average daily intake of			Final weight of kidneys		N.P.N. of blood
				P <sub>2</sub> O <sub>5</sub>	Al	C <sub>6</sub> H <sub>6</sub> O <sub>6</sub>	Right	Left	
		days	gm.	mgm.	mgm.	mgm.	gm.	gm.	mgm. per 100 cc.
727 ♀	C	292	13.0	33	8.9		0.7	0.8	45.4
755 ♀	C	297	14.7	37	10.1		0.8	0.8	45.0
769 ♀	C	304	13.6	34	9.4		0.8	0.9	53.3
771 ♀	C	307	14.5	36	10.0		0.8	0.8	43.7
773 ♀	C	303	13.7	34	9.4		0.7	0.8	44.8
		Average 13.9		35	9.6		0.8	0.8	46.4
730 ♂	D	247	14.7			214	1.0	1.0	47.0
731 ♂	D	252	15.3			222	1.0	1.1	43.5
742 ♂	D	285	15.4			224	1.1	1.0	44.0
746 ♂	D	285	15.4			224	1.0	1.0	42.6
747 ♂	D	248	14.9			217	1.0	1.1	45.7
748 ♂	D	285	14.7			214	1.1	1.1	42.8
750 ♂	D	252	15.5			225	1.1	1.1	43.8
752 ♂	D	253	16.1			234	1.0	1.1	43.6
757 ♂	D	285	14.8			215	1.1	1.2	46.1
759 ♂	D	288	15.9			231	1.1	1.2	44.3
760 ♂	D	288	15.6			227	1.1	1.2	45.1
775 ♂	D	288	14.8			215	1.1	1.1	45.7
778 ♂	D	288	14.5			211	1.0	1.1	45.3
783 ♂	D	288	14.0			203	1.0	1.0	45.5
784 ♂	D	248	15.9			231	1.1	1.2	43.2
786 ♂	D	288	16.4			238	1.1	1.1	45.4
		Average 15.2				222	1.1	1.1	44.6
729 ♀	D	297	12.7			185	0.7	0.7	43.4
736 ♀	D	297	12.6			183	0.8	0.8	45.5
743 ♀	D	295	12.7			185	0.7	0.7	44.4
745 ♀	D	295	12.4			180	0.7	0.7	44.3
751 ♀	D	301	14.0			204	0.9	0.9	44.6
761 ♀	D	299	13.0			189	0.7	0.7	44.0
762 ♀	D	299	13.0			189	0.8	0.8	43.4
766 ♀	D	292	14.6			212	0.8	0.9	45.4
768 ♀	D	307	14.5			211	0.9	0.9	51.2
770 ♀	D	301	13.7			199	0.9	0.9	47.8
776 ♀	D	303	12.5			182	0.7	0.7	44.2
780 ♀	D	299	13.6			198	0.8	0.8	42.9
782 ♀	D	295	14.0			204	0.7	0.8	42.9
		Average 13.3				194	0.8	0.8	44.9

protein nitrogen of the blood. Kidneys of 50 of the rats of both sexes selected at random from the four groups of the first generation, but including all of the second generation animals, were subjected to microscopic examination by Professor R. H. Jaffe of the Department of Pathology, College of Medicine, University of Illinois. Without any information as to the nature of the investigations, Professor Jaffe reported that with the exception of Rat 744, which evidently developed spontaneous nephritis, no differences were to be observed between the kidneys of the rats which received the baking powder diets and those upon the control ration. It is apparent, therefore, that evidence of deleterious effects was not revealed either by the chemical analysis of the blood or by the histological examinations of the kidneys.

TABLE III  
SECOND GENERATION OF RATS

Rat no. and sex	Diet no.	Duration of experi- ment	Average daily food con- sumption	Average daily intake of			Final weight of kidneys		N.P.N. of blood
				P <sub>2</sub> O <sub>5</sub>	Al	C <sub>4</sub> H <sub>10</sub> O <sub>8</sub>	Right	Left	
		days	gm.	mgm.	mgm.	mgm.	gm.	gm.	mgm. per 100 cc.
999♂	2B	72	17.1	416			1.0	1.0	56.6
1001♂	2B	76	15.8	384			0.9	1.0	44.1
1000♀	2B	72	14.4	350			0.9	0.9	50.8
1002♀	2B	76	14.1	343			0.8	0.8	50.1
		Average	15.4	373			0.9	0.9	50.4
1005♂	2C	56	15.5	78	21.3		1.0	1.0	40.7
1006♂	2C	56	15.7	79	21.6		0.9	0.9	43.2
1003♀	2C	56	14.0	70	19.2		0.7	0.7	41.9
1004♀	2C	56	13.9	70	19.1		0.8	0.8	44.3
		Average	14.8	74	20.3		0.9	0.9	42.5
1008♂	2D	52	14.9			433	0.8	0.8	44.3
1011♂	2D	52	14.7			427	0.8	0.9	42.6
1012♂	2D	52	14.1			410	0.7	0.8	44.3
1014♂	2D	52	14.1			410	0.9	0.9	43.2
1009♀	2D	52	12.9			375	0.6	0.6	45.1
1010♀	2D	52	12.9			375	0.7	0.8	43.5
1013♀	2D	52	11.4			332	0.6	0.6	43.0
1015♀	2D	52	12.4			361	0.6	0.6	44.9
		Average	13.4			390	0.7	0.8	43.9

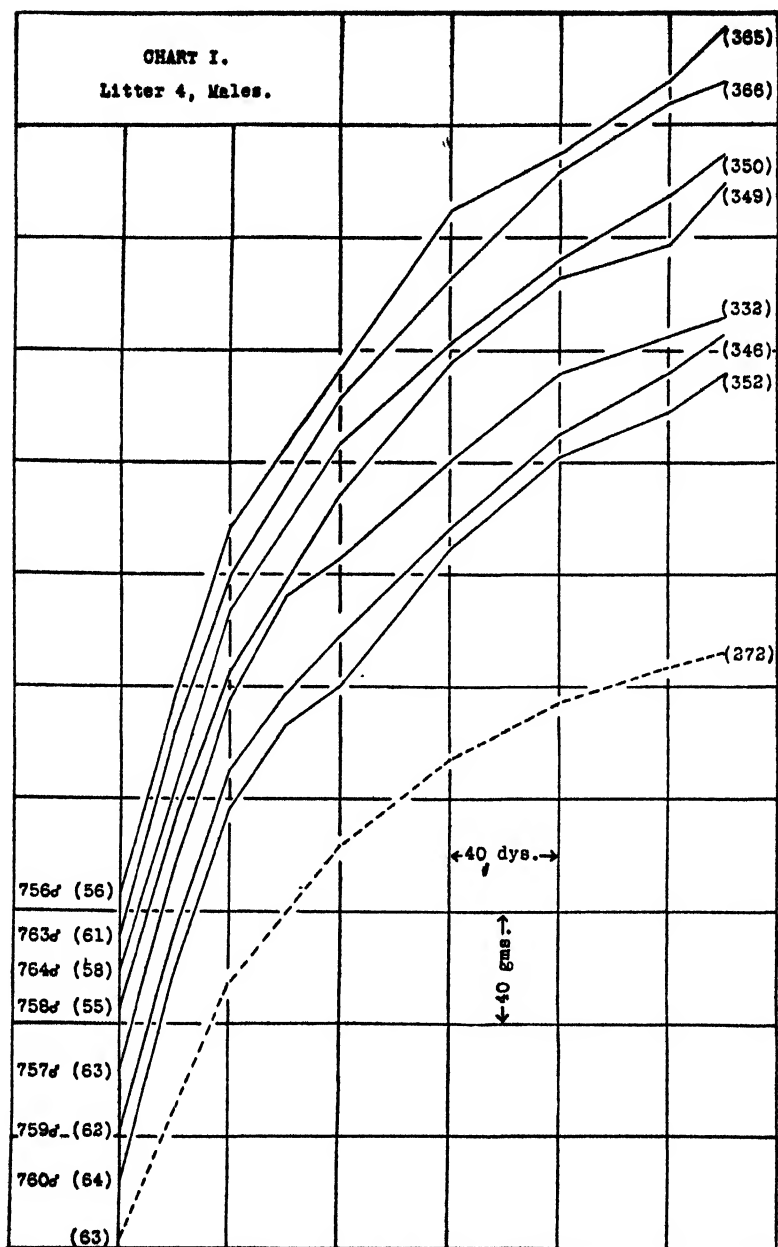


CHART I.

The numbers in parentheses denote the initial and final weights of the rats. The broken line curves show the so-called "normal" growth rate (Donaldson).

Rats 756, 763: Diet A, devoid of baking powder.

Rat 764: Diet B, phosphate powder.

Rat 758: Diet C, sodium aluminum sulfate powder.

Rats 757, 759, 760: Diet D, tartrate powder.

It has seemed unnecessary, in view of the uniformity of the data, to reproduce the growth curves of all of the first generation animals. In Charts I to III inclusive are presented the individual curves of 20 rats. These were selected because they belonged to litters the members of which were widely distributed upon the four diets. Inasmuch as many of the

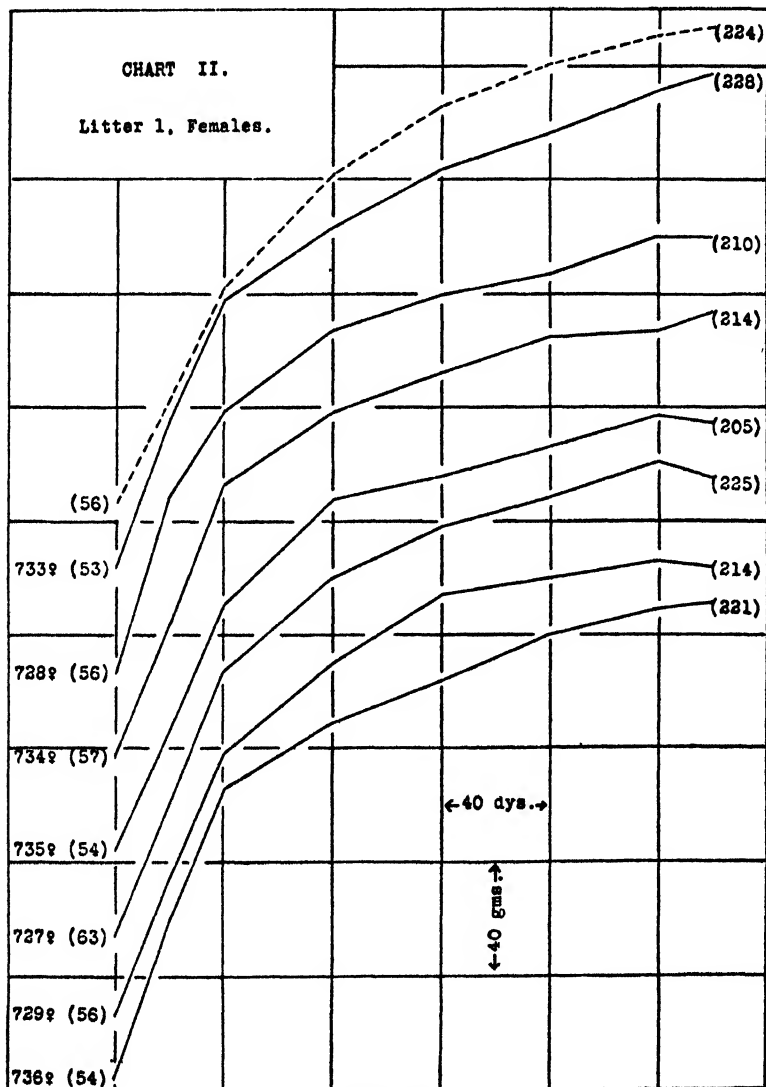


CHART II

Rats 733,728: Diet A, devoid of baking powder.

Rats 734,735: Diet B, phosphate powder.

Rat 727: Diet C, sodium aluminum sulfate powder.

Rats 729, 736: Diet Diet D, tartrate powder.

rats were bred after 220 days, the curves are shown for this period only. In Charts IV and V are presented composite curves for *all* of the first generation males and females respectively. In the latter charts the lines connect points representing the average weights at the beginning of the experiments and at the expiration of 220 days. Thus the average rate of growth of each group is indicated by the slope of the curve. This method of

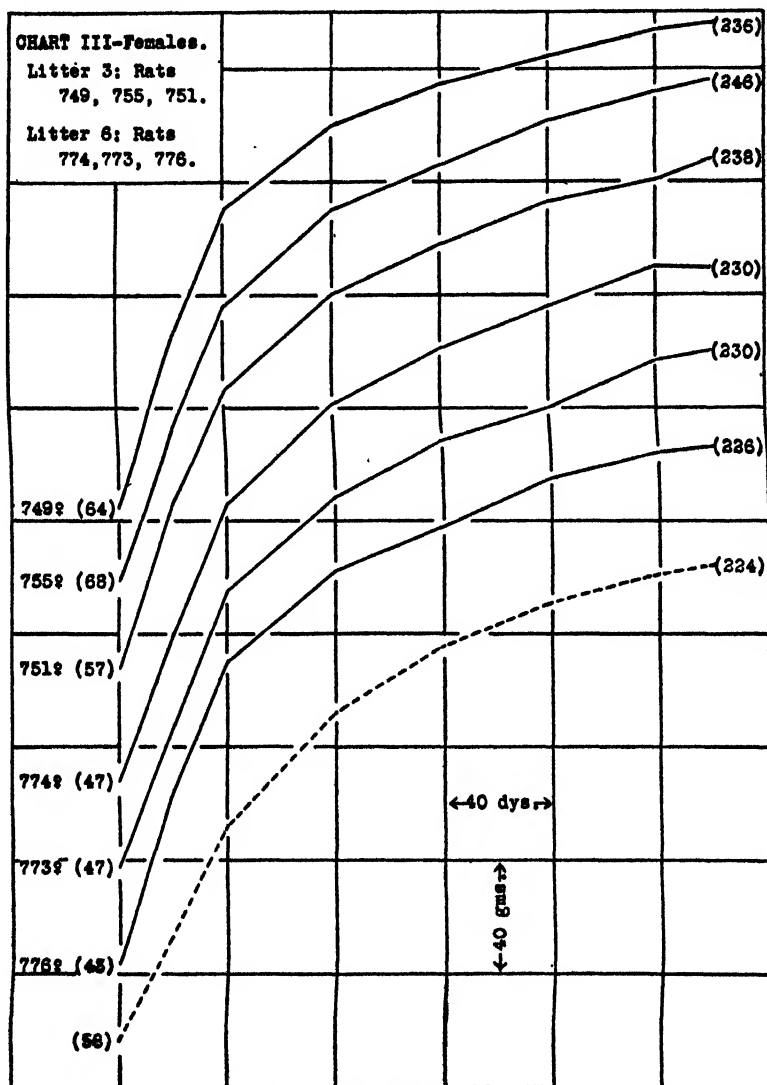


CHART III

Rats 749, 774: Diet A, devoid of baking powder.

Rats 755, 773: Diet C, sodium aluminum sulfate powder.

Rats 751, 776: Diet D, tartrate powder.

plotting appeared to afford the best opportunity of bringing out slight variations in the rates of increase in body weight. Both the individual and composite curves show quite clearly that the presence of the baking powders in the foods exerted no detectable influence upon the growth rate. All of the males (Chart I), and the females of Litters 3 and 6 (Chart III) grew more rapidly than the so-called "normal" of Donaldson (1924) for animals of the same size and sex. The females of Litter 1 (Chart II) increased in weight at almost exactly the rate indicated by the Donaldson data. The rats which received the phosphate powder (Diet B) grew slightly less rapidly than those of the corresponding sex upon the other three rations (Charts IV and V), but one would scarcely be justified in concluding that the differences are significant. Indeed, the growth data appear to afford additional proof of the fact that under the conditions of our experiments, neither of the baking powders exerted injurious effects. Similar conclusions apply to the experiments upon the second generation animals (Chart VI). With these, still more rapid growth was secured despite the presence of twice the quantities of baking powders given to the first generation animals.

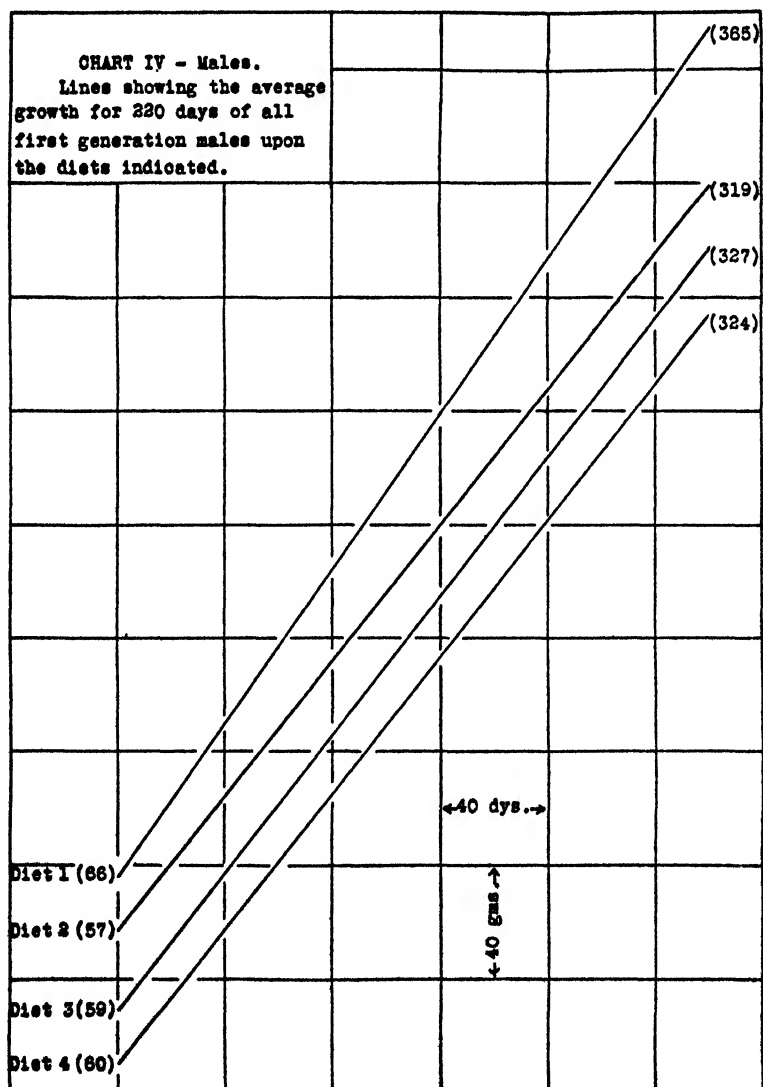
It should be emphasized that while the amounts of the leavening agents employed in Diets B, C and D conformed to the directions given on the baking powder containers, nevertheless the fact that the entire food supply contained the powders led indirectly to a very large intake. Thus for a 70 kilo man daily doses of the leavening agents comparable to those ingested by our first generation animals would amount to approximately 42 gm. of  $P_2O_5$  for the phosphate powder, 8.9 gm. of  $P_2O_5$  and 2.5 gm. of  $Al^1$  for the phosphate-aluminum powder, and 30 gm. of tartaric acid for the tartrate powder. Obviously, the results of such calculations must not be interpreted too literally, but at least they serve to demonstrate that the doses received by our animals were enormously greater in proportion to weight than could be obtained by the human subject in the use of baking powder breads.

In this connection reference should be made to the recent papers of Schaeffer and associates (1928) dealing with the physiological influence of a sodium aluminum sulfate-calcium acid phosphate baking powder. These authors list a formidable array of pathological findings which they attribute to the ingestion of the leavening agent. Among the alleged effects are retardation of gastric evacuation, intestinal ulceration, diarrhoea, in-

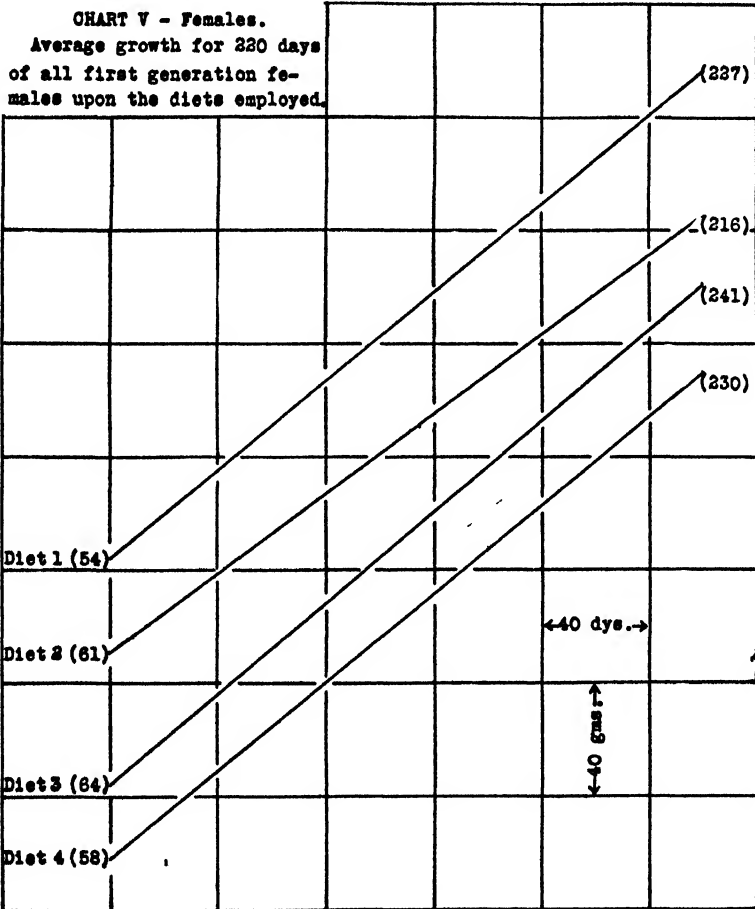
---

<sup>1</sup> The largest dose of aluminum employed by the Referee Board was 1000 mg. per day, equivalent to approximately 10 level teaspoonfuls of baking powder. The Board points out that such an intake could occur only if one subsisted almost entirely upon baking powder bread.

hibition in growth and atrophy of the female genital organs. As subjects for the experiments the authors employed chickens, dogs, rats and mice. They state that the diets were toxic for rats only when the latter had initial weights less than 90 gms. Heavier rats were resistant to the baking powder residues. It should be pointed out that all of our animals weighed less than 90 gm., and most of them less than 65 gm. when placed upon the baking powder diets. Probably the differences in findings in the two series of experiments are to be accounted for in part by the excessive amounts of baking powder employed by Schaeffer. Thus Diet A<sub>2</sub> (Schaeffer, *et al.*,



1928, pp. 53 and 60) which was used for the rat growth studies, was prepared by mixing 207 gm. of baking powder with 1385 gm. of the other dry materials. Such a mixture scarcely would be expected to promote normal growth. Possibly even a *necessary* inorganic dietary component like sodium chloride might inhibit growth if included in the ration at a 15 per cent level. However, despite the excessive proportions of baking powder in Schaeffer's diet, the rats receiving it succeeded in gaining



although at somewhat slower rates than the control animals. Under the circumstances the results would appear to emphasize the *non-toxic* nature of the leavening agent. It is to be noted also that in Schaeffer's control diet (Diet L<sub>2</sub>) bone meal replaced the baking powder of the experimental ration. The better growth which resulted suggests the possibility that the bone meal may have exerted a supplementing action. In any event the



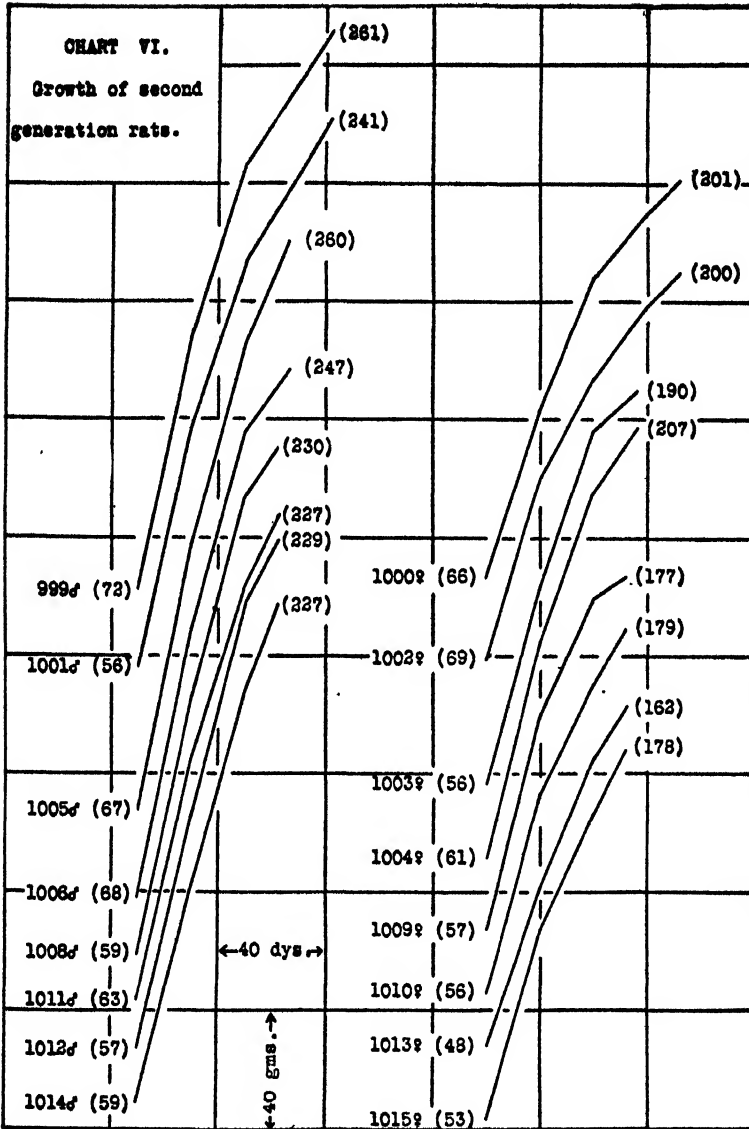


CHART VI

These animals received diets (designated in Table III as 2B, 2C, and 2D) containing double doses of the baking powders.

Rats 999, 1001, 1000, 1002: Diet 2B, phosphate baking powder.

Rats 1005, 1006, 1003, 1004: Diet 2C, sodium aluminum sulfate powder.

Rats 1008, 1011, 1012, 1014, 1009, 1010, 1013, 1015: Diet 2D, tartrate powder.

use of the meal introduced a variable other than the omission of the aluminum salt.<sup>2</sup> In not a single one of our experiments was there evidence of any of the abnormalities reported by Schaeffer. Our rats remained vigorous throughout the experiment, and at no time showed signs of alimentary disturbances. Nor were pathological alterations revealed at autopsy. We are convinced, therefore, that under the conditions employed by us the baking powders exerted no detectable deleterious action.

### SUMMARY

Growth experiments extending through two generations of rats have been conducted to determine whether baking powders exert deleterious effects. The results indicate that in the proportions ordinarily employed in the preparation of bread, and with twice these amounts, three typical baking powders in which the leavening agents are calcium acid phosphate, a tartrate, and a mixture of calcium acid phosphate and anhydrous sodium aluminum sulfate respectively, exert no detectable toxic or injurious action. The animals receiving the powders grew as rapidly and reproduced as satisfactorily as control rats upon a similar diet without baking powder. Analyses of the blood and histological examinations of the kidneys likewise failed to reveal evidence of injury.

### BIBLIOGRAPHY

- Donaldson, H. H., *The Rat*, Wistar Institute, Philadelphia, Second edition, 1924.  
Folin, O., and Wu, H., *Jour. Biol. Chem.*, 1919, XXXVIII, 81.  
Gies, W. J., *Biochem. Bull.*, 1916, V, 151.  
McCollum, E. V., Rask, O. S., and Becker, J. E., *Jour. Biol. Chem.*, 1928, LXXVII, 753.  
Myers, V. C., and Killian, J. A., *Jour. Biol. Chem.*, 1928, LXXVIII, 591; Myers, V. C., Mull, J. W., and Morrison, D. B., *ibid.*, 595; 605; 615; and 625.  
Pearce, R. M., and Ringer, A. I., *Jour. Med. Res.*, 1913, XXIX, 57.  
Post, W. E., *Jour. Am. Med. Assoc.*, 1914, LXII, 592.  
Referee Board of Consulting Scientific Experts, *U. S. Dept. Agric., Bull.* 103, 1914.  
Rose, W. C., *Jour. Pharm. Exp. Therap.*, 1924, XXIV, 123.  
Salant, W., and Smith, C. S., *Amer. Jour. Physiol.*, 1914, XXXV, 239.  
Schaeffer, G., Fontes, G., Le Breton, E., Oberling, C., and Thivolle, L., *Bull. Soc. Scientif. d'Hyg. Alim.*, 1928, XVI, 1-24 and 49-79.  
Schmidt, C. L. A., and Hoagland, D. R., *Univ. Calif., Pub. Path.*, 1919, II, 215.  
Underhill, F. P., Wells, H. G., and Goldschmidt, S., *Jour. Exp. Med.*, 1913, XVIII, 322.

<sup>2</sup> Since writing the above McCollum, Rask and Becker (*Bull. Soc. Sci. d'Hyg. Alim.*, 1929, xvii, 65) have published a criticism of the paper of Schaeffer and associates to which the latter (*Ibid.* 1929, xvii, 74) have replied.



---

# ROLLED OATS AND BRAN IN A SCURVY-PRODUCING DIET AND THE NEGATIVE CONTROL TEST

BY E. W. SCHWARTZE, F. J. MURPHY, AND R. M. HANN

With the assistance of William Waugh, Deborah  
D. Marsh, and Mary Marsh

*(From the Mellon Institute of Industrial Research,\*  
University of Pittsburgh, Pittsburgh, Pa.)*

Received for Publication—June 28, 1929

THE early investigators of experimental scurvy used simple diets containing raw materials, whereas later investigators, in an attempt to make their diets conform to the prevailing views of nutrition, modified their dietary standards with the consequent introduction of laborious, if not actually inadvisable, procedures. Owing to the necessity for the preparation of large quantities of a scurvy-producing diet, we have investigated this problem with the object of introducing a labor saving and tested standard procedure.

This paper relates largely to the use of a diet containing rolled oats and bran with butter fat and salts. This subject was studied, first, because it appeared that oats or bran or both had been practically universally used ever since the beginning of scurvy investigations; second, because these materials seemed to be of much better feeding quality than is usually ascribed to them; and third, because they are readily obtained and make up into a diet which is very easy to handle.

Although the principles underlying the necessity of a diet free from vitamin C but adequate in other respects are known to all, it should be noted that no one has yet shown any diet to be free from vitamin C. Moreover, little or no attention has been given to the quality of subject animals, with the result that the negative control tests, as used at present, could be presumed to throw light on the quality of animals rather than on the quality of the diet, but certainly not on both simultaneously. It is obvious, therefore, that until it is established how long certain standard animals should live on a diet proven to be free from vitamin C, investigators will continue to reason in circles.

Osborne and Mendel (1) showed that 10 per cent whole oat protein in

---

\* Contribution from the Utensil Fellowship.

the diet produced normal growth in rats, whereas the effect of 8 per cent was questionable. Smith and Hendricks (2) showed that rats grew at about one-third the normal rate over a course of 14 weeks when fed a diet containing 92.5 per cent of rolled oats, and concluded that the deficiency was due to a lack of the anti-pellagic fraction of the multiple vitamin B. Rolled oats contain about 16 per cent crude protein, very little material except the hull being removed in milling.

Osborne and Mendel (3) fed rats on bran at a 9 per cent crude protein level. Only one of four rats ate enough to grow well. Twenty per cent of the ingested nitrogen appeared in the feces instead of the more usual 10 per cent. At a 10 per cent crude protein level, growth of rats was exceedingly good for Murphy and Jones (4) during the first 100 days. The slight relative deficiency appearing later was made good with 5 per cent of patent flour. Jones and Gersdorf (5) found that bran is liberally supplied with all of the so-called essential amino acids. Bell and Mendel (6) found that 20 per cent of bran in the diet contained sufficient vitamin B complex to produce normal growth in mice, whereas 10 per cent was insufficient. Bran contains about the same amount of crude protein as rolled oats.

Hess (7) states that wheat germ possesses some antiscorbutic value. Jones and Gersdorf (5) found no evidence of germ in their sample of bran but estimated, on the basis of starch content, a possible 0.5 per cent.

Chick and Delf (8) used a diet of oats and bran and obtained good growth in guinea pigs fed with 30 grams of cabbage daily.

These references point to the good feeding qualities of oats and bran. Possibly, according to more modern standards, they may not be suitable for testing the optimum ability of animals to grow, but it would certainly be expected that guinea pigs should do very well indeed on a mixed diet containing the two, if supplemented with butter fat salts, and vitamin C.

#### PRELIMINARY PREPARATION OF ANIMALS

Owing to the difficulty of obtaining commercially large numbers of experimental animals, which were known to have had substantially the same treatment, and which could be presumed to be standard in any degree, it was deemed advisable to subject them to a preliminary treatment. They were observed for three weeks prior to the experiment. Individual growth and food consumption records were kept. Measured amounts of strained juice of canned tomatoes and fresh spinach were fed. The tomato juice doses were graduated upward from the second day and stopped three days before the animal was used in a scurvy experi-

ment. Twenty-five cc. of tomato juice were fed the second, third, and fourth days, 50 cc. on each of the next six days, and 75 cc. on the following five to eight days. No tomato juice was fed the first day or the last three days. Fifteen gm. of fresh spinach were fed each day.

In this way, by feeding an excessive amount of vitamin-C bearing material, it was possible for the animal to have a chance to retain considerable vitamin C and at the same time to reduce any deficiency, not apparent on purchasing, which might have occurred during the first few weeks of its life. Thus, by granting the same ability or lack of ability in each animal to store vitamin C, we have at the beginning of each experiment a set of animals reasonably standard as to freedom from and resistance against scurvy.

TABLE I  
EXPERIMENTAL DIETS PERCENTAGE OF INGREDIENTS

Diet	OB <sub>1</sub>	OB <sub>2</sub>	OB <sub>3</sub>	YOB	1/2YOB	OBG <sub>1</sub>	OBG <sub>2</sub>	Kenny*
Rolled oats	60.89	52.48	40.00	24.62	42.70	56.37	42.38	39
Bran	31.39	26.73	20.00	12.31	21.23	28.88	21.68	20
Butter fat	4.95	4.95	5.00	9.85	7.40	4.92	4.92	10
NaCl	.99	.99	1.00	.99	.98	.99	.99	1.0
NaH <sub>2</sub> PO <sub>4</sub> , H <sub>2</sub> O	.49	.84	1.65	—	.25	.54	1.47	—
CaCO <sub>3</sub>	1.49	16.34	1.60	2.95	2.22	1.33	1.47	—
Brewer's yeast	—	—	—	49.26	24.69	—	—	—
Corn starch	—	12.38	30.75	—	—	—	21.88	—
Calf skin gelatin	—	—	—	—	—	.69	.52	—
Skim milk powder	—	—	—	—	—	—	—	30
Crude protein	14.7	12.4	9.5	32.5	22.7	20.8	14.8	20.9
Ca/P Ratio**	.14/.16	.14/.16	.14/.16	.26/.28	.20/.22	.14/.16	.14/.16	.14/.16

\* This diet is described by Kenny (9).

\*\* Ratios are given in approximate amounts (gm.) of calcium and phosphorus in 20 gm. of food, which is a maximum daily ration.

The preliminary period of three weeks served another purpose in permitting us to weed out many of those animals which had incipient or latent disease. We rejected animals which did not show normal appetites and consistent growth and subjected them to a thorough post-mortem examination. Chronic pulmonary disease or some other pathologic condition was usually found. Only experiments on male animals are reported.

#### THE DIETS

The composition of the diets is given in Table I. The purposes of these diets are obvious, namely, to study the effect of reduction of protein in the OB<sub>1</sub> diet, which contained the maximum amount of crude protein possible when using only oats and bran, as well as its possible supplementation

by other protein. In addition, the OB<sub>1</sub> diet listed in the first column of the table was modified and fed in a liquid or mushy state (Diet LOB) containing 20 per cent of solids and 80 per cent of tap water, or (Diet LOBTJ) containing 20 per cent of solids, 65 per cent of tap water, and 15 per cent of filtered juice of canned tomatoes. These two modifications were used to test the effect of cooking in the presence of alkali.

The LOB diet was prepared by mixing the oats, bran, and water in a large flask. Sufficient sodium hydroxide was added as N/2 solution to form the necessary amount of sodium chloride for the diet on later neutralization with hydrochloric acid. This mixture was placed in an oven held at 95° C for 2 hours, on each of three consecutive days, without causing gelatinization of starch to such an extent as to prevent ease of shaking and mixing, the flask being stoppered with a cotton plug. Between heatings the material was kept in a refrigerator to prevent spoiling. After the third heating sufficient standard hydrochloric acid was added to neutralize the sodium hydroxide present. The calcium carbonate and monosodium phosphate were also added at this point. The mixture was then cooked at the temperature of boiling water for one hour to gelatinize the starch, the melted butter-fat was added, and the diet was thoroughly mixed. This diet was fed in a negative control experiment, supplemented by brewer's yeast, and in a positive control experiment supplemented by yeast and 15 cc. of tomato juice daily. The yeast was fed by pipette as a suspension in water, 400 mg. being given daily for the first 17 days of the negative control experiment. The tomato juice was the filtered juice of canned tomatoes and was fed by pipette throughout the positive control experiment.

The LOBTJ diet was prepared exactly as the LOB diet except that 15 cc. of filtered tomato juice were used to replace a like amount of water per 100 gm. of final diet prepared. The tomato juice was added to the first mixture before any heating was done. This diet was used in a negative control experiment supplemented by yeast, as mentioned above, to ascertain whether the method of cooking caused destruction of vitamin C when the latter was known to have been present.

### RESULTS OF FEEDING TESTS

Two sets of data were obtained, namely, those dealing with the sufficiency of these diets when fed with 15 cc. of filtered juice of canned tomatoes daily (positive controls) and when no tomato juice was fed (negative controls).

TABLE II  
POSITIVE CONTROLS ON VARIOUS DIETS

Animal No.	Entering weight	Final weight	Total gain	Duration	Total food eaten	Gain per gm. food	Gain per gm. protein	Gain per day
Male	gm.	gm.	gm.	days	gm.	gm.	gm.	gm.
OB <sub>1</sub> Diet—14.7 per cent Crude Protein*								
620	275	496	221	85	1709	.129	.880	2.6
768	235	357	122	50	740	.165	1.12	2.4
770	225	405	180	60	1118	.161	1.09	3.0
771	300	424	124	51	921	.135	.934	2.4
779	211	332	121	60	815	.148	1.00	2.0
Average	249	403	154	61.4	1061	.148	1.01	2.5
OB <sub>2</sub> Diet—12.4 per cent Crude Protein*								
763	236	363	127	48	910	.139	1.12	2.6
767	252	468	216	60	1212	.179	1.44	3.6
769	259	376	117	60	999	.117	.94	2.0
776	249	363	114	51	861	.132	1.07	2.2
Average	249	392.5	144	55	995	.142	1.14	2.6
OB <sub>3</sub> Diet—9.5 per cent Crude Protein*								
803**	244	325	81	41	721	.112	1.18	2.0
YOB Diet—32.5 per cent Crude Protein*								
616†	320	603	283	90	2214	.127	.393	3.1
One-Half YOB Diet—22.7 per cent Crude Protein*								
618†	248	559	311	90	1787	.174	.767	3.5
OBG <sub>1</sub> Diet—20.8 per cent Crude Protein*								
765**	221	348	127	51	818	.155	.746	2.5
OBG <sub>2</sub> Diet—14.8 per cent Crude Protein*								
764	267	444	177	60	1128	.157	1.06	3.0
773	274	406	132	60	1036	.127	.86	2.2
775	250	410	160	50	915	.175	1.18	3.2
Average	264	420	156	56.7	1026	.152	1.03	2.7
Milk Powder—Rolled Oats—Bran Diet—20.9 per cent Crude Protein‡								
Average of 11 animals	329	626	297	102	2131	.138	.66	2.9

Notes to table on next page.



The positive control experiments with the OB<sub>1</sub> and OB<sub>2</sub> diets, containing 14.7 and 12.4 per cent of crude protein respectively, show that these diets are capable of producing good growth in the guinea pig. For comparison a summary is given of data obtained from the feeding of a diet of rolled oats, bran, and skim milk powder containing 20.9 per cent of crude protein, as well as from two experiments in which yeast furnished large amounts of protein. One experiment with 9.5 per cent of crude protein obtained from rolled oats and bran indicates the possibility of even this concentration being adequate. A higher concentration of protein than can be attained by using rolled oats and bran, may be expedient if one wishes to use as subjects in scurvy experiments animals whose growth capacity is being stimulated to a maximum or which have unquestionably had the best opportunity to grow. While it would seem possible for it to be only a matter of experimenter's choice, we propose, nevertheless, to consider the advisability of this supplementation in another paper.

The two experiments with dried brewer's yeast indicate that guinea pigs can do well on as much as 12 to 25 times the amount of yeast which would be supposed to furnish the optimum amount of vitamin B for a rat. Yeast, however, is not an advantageous source of protein for guinea pigs on account of their objection to its taste when used in the greater concentrations.

The experiments with gelatin as a supplement were inconclusive. Rather than attempt to work further on this intricate and involved point, we have decided to avoid this laborious task and to direct our attention in the future to protein-bearing material which when taken alone is known to be entirely adequate for nutrition.

Our experiments, like all experiments which are performed on guinea pigs, throw no final light on the question of vitamins other than the anti-scorbutic factor, since these principles are furnished simultaneously with vitamin C in green food, etc. We have but one course of action at present and that is to furnish all factors except vitamin C in apparent super-abundance.

The results of negative control experiments are given in Tables III and IV. One important result from these experiments is not indicated

---

\* 15 cc. of tomato juice per day carried 0.1 gm. of crude protein (0.11% N).

\*\* Only one experiment each on OB<sub>2</sub> and OBG<sub>2</sub> diets is reported as the other animals started had to be discontinued on account of respiratory disease. It became evident as the experiment progressed that their replacement would not make any essential addition to the theme of this paper.

† Only one experiment in each group is reported on account of inability to get animals to consume the yeast diets.

‡ Vitamin C furnished by 15 gm. of fresh spinach daily.

directly in the table; namely, that these tests are absolutely unreliable unless performed on animals in the best of health and condition. It is possible to be deceived, since weakling, under-vitaminized and diseased animals, when fed diets which according to our modern criteria contain traces of vitamin C, will die in the interval set for healthy animals fed diets believed to be free or practically free of vitamin C. Every investigator finds himself in this dilemma, especially when using commercial animals. Since this is the case, it is obvious that the negative control test

TABLE III  
NEGATIVE CONTROLS ON VARIOUS DIETS

Animal No.	Preliminary period	Entering weight	Beginning Experimental weight	Maximum weight*	Final weight	Lived less than	Remarks
Male	days	gm.	gm.	gm.	gm.	days	
OB <sub>1</sub> Diet—Small Animals							
615	17	264	304	342(16)	210	27	
619	17	237	278	346(15)	175	33	
621	17	249	294	368(18)	184	29.5	
622	17	236	324	354(18)	188	32.5	—Small patch of atelectasis in both apices
624	17	262	338	353(16)	175	28.5	
625	17	242	314	327(10)	205	28.5	
628	17	245	291	355(13)	175	28.5	
627	17	247	270	369(14)	192	29	—Small patch of atelectasis
629	17	230	228	298(12)	175	26	
Average		246	293	346	187	29.2 ± 2.0	—40% loss from beginning experimental weight.
OB <sub>1</sub> Diet—Large Animals							
602	22	326	490	514(18)	275	34	
605	22	330	426	436(11)	218	28.5	
607	22	355	465	495(14)	274	29	
608	22	384	458	471(14)	239	31	
609	19	341	442	448(13)	212	28	
610	19	342	432	466(18)	245	30	
611	19	353	402	433(14)	240	30.5	
612	19	360	462	456(15)	244	32.5	—Small patch of atelectasis
613	19	330	462	471(15)	238	30	
614	19	342	400	415(14)	213	29	
Average		346	444	460	240	30.2 ± 1.8	—45% loss in beginning experimental weight.

\* Numbers in parenthesis refer to day of obtaining maximum weight.

does not serve two purposes simultaneously, namely, that of a test of quality of animals and that of quality of feeding materials.

The proper future and practical application of this test will undoubtedly take cognizance of rigid minimum as well as maximum survival times. Finding the absolute definition of a diet free from vitamin C, it would be expedient to select those raw materials which as purchased are likely to be most free from this vitamin, or, in the conventional terms of the day, are free from detectable amounts of it.

Two sets of animals were used on the negative control experiment or

TABLE IV  
NEGATIVE CONTROLS ON VARIOUS DIETS

Animal No.	Preliminary period	Entering weight	Beginning experimental weight	Maximum weight*	Final weight	Lived less than	Remarks
Male	days	gm.	gm.	gm.	gm.	days	
LOB <sub>1</sub> Diet, Cooked in Alkali							
737	21	275	376	416(11)	218	31	400 mg. of yeast daily for first 17 days of the experiment by pipette.
745	21	285	371	413(6)	237	22.5	
740	21	286	378	416(14)	255	27	
743	21	283	377	430(16)	236	30	
Average		282	375	418	236	27.6	Average loss in beginning experimental weight 27 per cent.
Full and One-Half Yeast Diets (YOB and 1/2 YOB)							
604	22	326	366	356(18)	242	29	Appetite fair to good. YOB diet.
599	22	308	327	350(21)	161	30	Refused YOB. Fed 1/2 YOB. beginning 4th day.
626	17	248	302	374(18)	180	31.5	1/2 YOB diet.
Average		294	332	360	194	30.2	Average loss in experimental weight 48 per cent.
Gelatin (OBG <sub>1</sub> ) Diet							
643	22	278	371	383(7)	192	26	Six other rejected animals showing chronic lung disease lived on an average of 27.75 days.
669	22	285	370	406(16)	200	32	
670	22	280	334	352(14)	199	27	
Average		281	358	380	197	28.3	Average loss in experimental weight 45 per cent.

Numbers in parenthesis refer to day of obtaining maximum weight.

the OB<sub>1</sub> diet. The larger animals, weighing 400–500 gm., showed a survival time of 28 to 34 days with a mean of 30.2 days and a standard deviation (10) of  $\pm 1.8$  days. The smaller animals, weighing 250–350 gm., showed a survival time of 26 to 33 days with a mean survival time of 29.2 days and a standard deviation of  $\pm 2.0$  days.

We cannot say but that the survival times might ordinarily be slightly shorter by a day or so than we have found, if cumulation is shown to be a factor. It is improbable that experimenters would obtain animals which had had an opportunity to acquire vitamin equal to that of animals herein referred to, although it is still possible that cumulation may not be a factor.

The series of experiments with the LOB<sub>1</sub> diet cooked in alkaline solution was not a complete success on account of the rejection of many animals having lung disease, which escaped diagnosis in our preliminary scrutinizing examination. Only four unrejectable experiments were obtained. One of these is apparently of rather short duration and points to the absolute necessity for more numerous data. If this experiment be omitted, the average duration of life of the other three is 29.3 days, almost a perfect check with the animals of the same size on the uncooked OB<sub>1</sub> diet. Many of the rejected experiments were of a satisfactory duration but others were not, so we have followed the procedure of reporting no experiments on animals with certain lung disease. The several experiments without yeast for controlling the destruction of the antineuritic vitamin, those for controlling the effect of alkali on the protein, as well as those designed to show that the method was susceptible of destroying added vitamin C, are not reported. The results were satisfactory enough, on the whole, to indicate that the test was fair and that there was at least no appreciable vitamin C in our rolled oats and bran. A similar series of tests will be run again later on the rolled oats-bran diet, supplemented with other protein.

The three negative control experiments with yeast are in agreement with the supposition of Cohen and Mendel (11) as well as with Hess (7) that our sample of yeast contained no detectable amounts of vitamin C. Yeast<sup>†</sup> has also been used in scurvy diets by Parsons and Reynolds (12) and by<sup>†</sup> Bertha Clow\* with apparent success. We fed 12 to 25 times the amount that ordinarily might be used for furnishing vitamin B to a rat, and we believe that a few experiments of this type are much more conclusive for showing up traces of vitamin C than more numerous ones in which only the<sup>†</sup> amount of yeast to be used regularly is administered.

Gelatin, which from its method of manufacture by the lime water pro-

\* Personal communication to the authors.

cess, would not be expected to contain vitamin C, was found not to contain detectable traces of this factor.

#### SUMMARY

A diet containing 14.7 per cent of crude protein, obtained from rolled oats and bran in the ratio of 2:1, butter fat and salts, has been described. This is about the maximum amount of dietary protein possible to obtain by using these raw materials. It would appear that this diet is perfectly satisfactory for the nutrition of guinea pigs, and that it contains considerably more than a minimum of the known essential amino acids. The survival time on this diet alone of guinea pigs standardized by previous preliminary feeding of large amounts of vitamin C, has been found to be 28 to 34 days (average  $30.2 \pm 1.8$ ) for animals weighing 400–500 gm., and 26 to 33 days (average  $29.2 \pm 2.0$ ) for animals weighing 250–350 gm.

Some data obtained with this diet, when it was cooked in alkaline solution previous to feeding, indicate that it contains no detectable amount of vitamin C. It appears not improbable that it contains absolutely none.

A few preliminary experiments with dried brewer's yeast in large concentrations indicate that it is possible to use this material as a bearer of vitamin B in scurvy diets without fear of adversely affecting the guinea pigs or without the introduction of detectable amounts of vitamin C. We would not recommend it in large concentrations as a protein supplement on account of the taste.

The diet of oats and bran would appear to be eminently suited for animals placed in acute qualitative tests of materials which contain only traces of, or no, vitamin C. It would probably serve for the longer 90 day tests except that, for the present, expediency rather than demonstrable necessity would dictate that we supplement it with both protein and vitamin B complex. In view of the difficulty and uncertainty of obtaining final convincing evidence that would entirely suffice, it will be better in the future to direct attention to such supplementation.

Attention has been called to the unreliability of the negative control test and the possible fallacies in the results therefrom. It would be well for the investigator, if he is inclined even by tacit implication to insist on a diet free of vitamin C, to avoid the dilemma of being unable to prove from his tests whether the diet or the animals he is using are satisfactory. It is possible that at an early date this test may revert to its proper status, namely, one of animal rather than dietary control. Even though it is logically to be presumed that all commercial rolled oats, bran, and dried brewer's yeast are free of disturbing amounts of vitamin C, we would nevertheless

recommend a test of such materials before using as well as the preparation of the diets from large uniform batches of raw materials.

#### REFERENCES

1. Osborne and Mendel, *Jour. Biol. Chem.*, 1920, XLI, 275.
2. Smith and Hendricks, *Public Health Reports*, Feb, 5, 1926, 201.
3. Osborne and Mendel, *Jour. Biol. Chem.*, 1919, XXXVII, 557.
4. Murphy and Jones, *Jour. Biol. Chem.*, 1926, LXIX, 85.
5. Jones and Gersdorf, *Jour. Biol. Chem.*, 1925, LXXXIV, 241.
6. Bell and Mendel, *Amer. Jour. Physiol.*, 1922, LXXXII, 145.
7. Hess, *Amer. Jour. Dis., Child.*, 1917, XIII, 98.
8. Chick and Delf, *Biochem. Jour.*, 1919, XIII, 199.
9. Kenny, C. L., Diss. Columbia University, New York City, 1926.
10. Burgess, *The Mathematics of Statistics*, New York, 1927.
11. Cohen and Mendel, *Jour. Biol. Chem.*, 1918, XXXV, 425.
12. Parsons and Reynolds, *Jour. Biol. Chem.*, 1924, LIX, 732.



---

## NERVE DEGENERATION RESULTING FROM AVITAMINOSIS A

By J. S. HUGHES, H. F. LIENHARDT, AND C. E. AUBEL.

(From the Department of Chemistry, Kansas State Agricultural  
College, Manhattan)\*

Received for Publication—June 29, 1929

**B**ERI BERI, scurvy, and rickets were each recognized as a definite disease characterized in each case by well known symptoms and pathological lesions, long before the corresponding vitamins, vitamins B, C, and D, were discovered. The discovery of these vitamins served merely to clear up the etiology of these well known diseases. Very little information concerning the symptoms and pathology of these diseases has been added since it was discovered that they resulted from the lack of these vitamins.

This is not the case, however, with vitamin A. This vitamin was discovered as a result of the study of an eye disorder which was found to develop in experimental rats on a somewhat restricted diet. Aside from this eye trouble which occurred in experimental rats, no definite disease was known for which this vitamin could be considered as the etiological factor. For this reason it has been necessary to study the tissue changes which result when vitamin A is lacking in the diet. A number of investigators have reported the results of their studies of this subject.<sup>1</sup> The most extensive of these studies have been reported by Mori (1922) and Wolbach and Howe (1925).

The outstanding pathological change reported by these workers seems to be a keratinization of the epithelium throughout the body. This change in the epithelium lowers the resistance to bacterial infection, which results in secondary lesions. As a general rule in the last stages of avitaminosis A these secondary lesions, resulting from infection, overshadow the primary changes brought about as a direct result of the lack of the vitamin. In the rat the eye and surrounding tissues are almost always involved.

In no case, so far as we are aware, has anyone reported the finding of pathological changes in the nervous system accompanying avitaminosis A. Wolbach and Howe (1925) have the following to say concerning the influence of the lack of this vitamin on the nervous system in the rat.

"The Nervous System—As the rats exhibited no symptoms pointing to nervous system lesions, the peripheral nerves were not studied. No les-

---

\* Contribution No. 144.

<sup>1</sup> A good review of this work can be found in "The Newer Knowledge of Nutrition" (third edition) by McCollum and Simmonds.



ions were found in the brain, cerebellum and sympathetic ganglia. The ganglion cells of the myenteric plexus were normal whenever found."

While it is true that rats show no striking nervous symptoms accompanying avitaminosis A, it is not uncommon for those in the advanced stage of the disorder to show a distinct incoordination which suggests an involvement of the nervous system. This incoordination is very slight, however, in the case of rats. With swine, we have found the incoordination to be the outstanding symptom of avitaminosis A, while the eye lesions, which are so prominent in the rat, are of little importance.

In our studies (1928) of the importance of vitamin A for swine, six lots, including twenty-seven individuals, have been fed a ration which is deficient in vitamin A. This ration consisted of white corn, tankage, and bone ash. Complications from rickets were avoided by allowing the pigs an outside yard where they received an abundance of direct sunshine. The pigs were placed on this vitamin A-deficient diet at weaning time and were continued on the diet until they died, or until they reached such an advanced stage of the disorder that they would have died in a few days, when they were killed for post-mortem examination. As it was thought that the pigs would develop eye lesions similar to those which characterize avitaminosis A in rats and chickens, careful watch was kept of their eyes. While there was more or less watering of the eyes, there were no cases of severe conjunctivitis, and in no case did there seem to be an involvement of the tissue around the eye. In one case a small corneal ulcer developed. Without exception these pigs developed a marked nervous disorder, in from six to ten months, characterized by blindness, incoordination and spasms (Figure 1).

Hart, Miller, and McCollum (1916) observed similar symptoms and nerve degeneration in pigs, in their studies on the nutritive deficiencies of wheat and grain mixtures. Since their work was done before there was a clear understanding of the cause of deficiency diseases, they attributed the pathological condition to some toxic principle in the wheat rather than to a deficiency in the diet. The beneficial action of alfalfa in preventing these symptoms was ascribed to the amount of fat-soluble A and minerals which it contained. These substances in some way offset the toxic action of the wheat.

That this nervous condition in our experiment resulted from a lack of vitamin A was shown by the fact that it could be prevented or relieved by incorporating in the feed an adequate supply of substances known to be good sources of this vitamin, such as cod liver oil, butterfat, yellow corn, and alfalfa leaf meal.

As these symptoms indicated an involvement of the nervous system, histological examinations were made of the tissue. The tissue, after being fixed by Mueller's fluid, was stained with osmic acid, employing the Marchi method. Definite degeneration of the nerve bundles was found in the optic thalamus, optic, femoral, and sciatic nerves, and certain parts of the spinal cord. Nerves from normal hogs were examined by the same method using the same solution and in no case did they show suspicious staining characteristics. The accompanying micro-photographs illustrate the type of



Figure 1.—Pigs in an advanced state of avitaminosis A, a condition characterized by extreme incoordination and spasms.

degeneration found in the nervous tissue (Figure 2). These histological findings, together with the clinical symptoms, show quite clearly that the lack of vitamin A results in the degeneration of the nervous system in the case of swine.

Other animals show similar marked nervous symptoms in the advanced stages of avitaminosis A. In studying the importance of vitamins in the ration of dairy cattle, we have fed three mature cows on a feed deficient in vitamin A<sup>2</sup>. While these cows did not develop spasms like the pigs on a similar feed, they did show marked stiffness and incoordination.

Young chicks resemble pigs in the severity of the nerve disorder accompanying avitaminosis A. In one group of five hundred day-old chicks placed on a vitamin A-free feed, more than ninety per cent developed symptoms showing severe nerve degeneration<sup>3</sup>. One not familiar with the fact that

<sup>2</sup> Unpublished data.

<sup>3</sup> Unpublished data.

lack of vitamin A causes nervous symptoms in chicks, might mistake the condition for polyneuritis resulting from the lack of vitamin B (Cruckshank, Hart, and Halpin, 1928). Histological examination is now being made on the nervous system of these chickens to see whether or not they show the same type of nerve degeneration found in pigs.

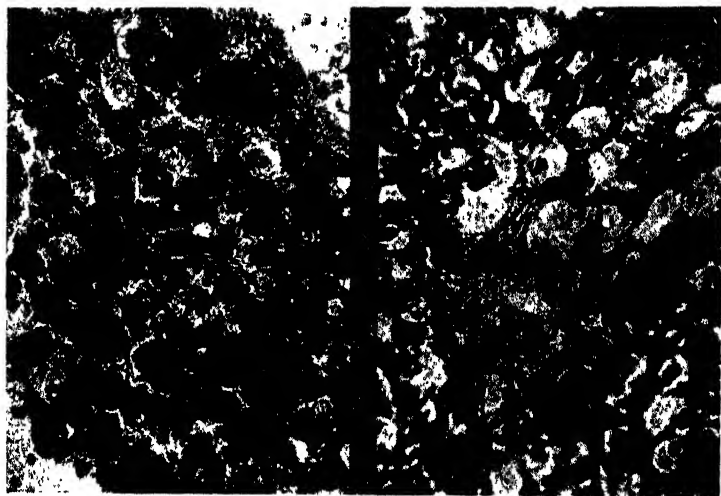


Figure 2.—Micro-photograph of cross section of spinal cord. Right hand figure, from normal cord. Left hand from cord of pigs in advanced stages of avitaminosis A. Blackened areas indicate degeneration.

### CONCLUSIONS

The lack of vitamin A in the diet of pigs, chickens, and cows, results in marked nervous symptoms characterized by impaired vision, incoordination and spasms.

Histological examination of the nerves of pigs in the advanced stage of avitaminosis A, shows degeneration of some of the nerve bundles. This degeneration was observed in portions of the spinal cord, optic, sciatic, and femoral nerves.

### BIBLIOGRAPHY

1. Cruckshank, Ethel M., Hart, E. B., and Halpin, J. G., 1928, *Poultry Science*, VII, 9.
2. Hart, E. B., Miller, W. S., and McCollum, E. V., 1916, *Jour. Biol. Chem.*, XXV, 239.
3. Hughes, J. S., Aubel, C. E., Lienhardt, H. F., *Technical Bulletin* 23.
4. Mori, S., 1922, *Johns Hopkins Hosp. Bul.* XXXIII, 357.
5. Wolbach, S. Burt, and Howe, Percy R., 1925, *Jour. Expt. Med.*, XLII, 763.



## INFLUENCE OF FIBER ON NITROGEN BALANCE AND ON FAT IN THE FECES OF HUMAN SUBJECTS

BY JESSIE WHITACRE\*, ALICE WILLARD\*\* AND KATHRINE BLUNT

*(From the Department of Home Economics, University of Chicago.)*

Received for Publication—July 3, 1929

DOES the presence of fiber in varying amounts in the ordinary diet affect the utilization of the nutrients? This question presented itself to the authors of this article when comparison was made of the results of two balance experiments with human subjects (1, 2) done in the Nutrition Laboratory of the University of Chicago. The two junior authors (A. W. and J. W.) each served as a subject in her own experiment, and one of them (J. W.) was a subject also in the other's study. The experimental diets, planned to meet present day criteria of adequacy, contained liberal, though not unusually large, amounts of fiber. The coefficient of digestibility of nitrogen in these two studies was found to be distinctly and consistently lower than the accepted average figure for mixed diets, (85 to 87 in one case and 88 to 89 in the other *vs.* the usual 92), while the coefficient of digestibility for fat, as determined in one of the Chicago experiments, even exceeded the accepted average figure (97.4 to 98.2 *vs.* the accepted 95). Also, of the two Chicago diets the one with the higher fiber gave the lower figure for the nitrogen.

The influence of fiber on the utilization of nutrients may have significance in problems of animal feeding as well as in human nutrition.

### PREVIOUS WORK

The extensive literature, which has accumulated on the subject of the digestibility of foods, contains comparatively little comment upon the influence of fiber on the utilization of the nutrients. But fiber has usually been included in experimental diets whether they were very simple, as in earlier days, or more complex, as in recent times.

In the numerous and valuable studies of the digestibility of particular foods made in the U. S. Department of Agriculture, the diets were deliberately kept very simple in order that the effect of the specific food might be apparent. For example, one may mention the studies of Woods and Merrill

\* Now on the staff of the Division of Rural Home Research in Texas Agricultural Experiment Station.

\*\* Now on the staff of the Department of Household Science, University of Toronto.

(3) with bread in 1900; Doane (4) with cheese in 1911; Langworthy and Holmes (5, 6, 7, 8) with certain animal fats in 1915, with grain sorghums and with very young veal in 1916, with certain vegetable fats in 1917; Holmes (9, 10) with some nut oils in 1918, and with wheat bran 1919; Langworthy and Deuel (11) with raw corn, potato, and wheat starches in 1920. The simplicity of food combinations used in the government studies served as a pattern for other investigators.

More recently the tendency in digestibility and balance experiments has been to employ a greater assortment of food materials. Typically varied experimental diets are those used by the authors of this report, by Rose and McLeod (12, 13) in their study of the digestibility of raw egg white, and by McLaughlin (14), in her comparison of spinach with milk as a source of calcium.

#### UNIVERSITY OF CHICAGO STUDIES—EXPERIMENTAL DIETS

The make-up of the diets in the University of Chicago studies, in the matter of the distribution of nitrogen and calories, and with respect to details of similarity and difference between the two diets, is shown in Table I.

The whole milk powder in J. W.'s diet was diluted so as to be the equivalent of cow's whole milk. In period 1 for A. W.'s diet, the milk was fresh pasteurized whole milk; in period 2, evaporated milk diluted to be the equivalent of cow's whole milk.

Calories provided by J. W.'s diet were approximately 2300 per day and 1945 in A. W.'s meals. Fuel values in J. W.'s were calculated from the percentage compositions found by analyses, and the calorific factors, protein 4.35, fat 9.45, carbohydrate 4.1, were not reduced for loss in digestion, since coefficients of digestibility were determined in this study. Fuel value for A. W.'s diet was calculated from figures in Sherman's tables (15).

The daily intake of the two subjects on A. W.'s diet varied only in that J. W. ate 100 grams of lettuce and 300 grams of peaches, while A. W. had but 30 grams of lettuce and 240 grams of peaches.

#### EXPERIMENTAL PROCEDURE

Each experimental period covered six days. Except for water and salt, all food portions eaten during each period were carefully weighed on a trip scales, and the food was consumed as nearly quantitatively as possible.

Samples of the food eaten on the last three days, also urine and feces for the same period, were analyzed.

For determination of nitrogen in urine, the micro-Kjedahl method of

TABLE I  
DISTRIBUTION OF NITROGEN AND CALORIES IN THE DAILY DIET

Foods	A.W.'s Diet						J.W.'s Diet					
	Period 1			Period 2			Period 1			Period 2		
	Intake Gm.	N. gm.	Total Energy	N. gm.	Total Energy	Calories	Intake gm.	N. gm.	Total Energy	Calories	N. gm.	Total Energy
Beef	100	3.14	155	3.17	155		135	4.73	161		4.47	202
Milk	133	0.74	91	0.78	91							
Dry Milk†							81	2.94	441		3.40	444
Bread	185	2.32	485	2.36	485		135	1.88	364		1.74	366
Potato	360	1.11	300	1.28	300		400	1.06	424		1.39	424
Butterfat	40	—	360	—	360		63	—	595		—	595
Sugar	8	—	32	—	32		40	—	164		—	164
Lettuce	100	0.12	19	0.13	19							
Celery							170	0.33	39		0.20	27
Apple, (pared)							300§	0.11	195		0.10	162
Peaches (canned)	300	0.24	138	0.23	138							
Orange juice	100	0.13	45	0.13	45							
Cereal (wheat endosperm)	28	0.50	100	0.50	100							
Bacon	35	*0.59	220	*0.59	220							
Totals		8.89	1945	9.17	1945			11.05	2383		11.30	2384
											11.41	2264

† Spray process.

§ 100 grams eaten raw; 200 grams cooked.

\* Calculated from Sherman.

Koch and McMeekin (16) was used, and a modification of it (17) for nitrogen in food and feces. In J. W.'s experiment ether-soluble substances in meat, potatoes, apples, celery, and feces were determined by the Soxhlet extraction method; in bread by the tentative A.O.A.C. method (18); and in the powdered whole milk by Jephcott's modification (19) of the Werner-Schmidt method. Coefficients of digestibility were calculated by the common equation:

$$\frac{\text{grams ingested} - \text{grams in feces}}{\text{grams ingested}} \times 100 = \text{percentage absorbed.}$$

### RESULTS AND DISCUSSION

The course of nitrogen and fat metabolism is shown in Table II. In each period both subjects were in practical nitrogen equilibrium. Approximately one gram of protein per kilogram of body weight per day had been eaten in each case.

The figures for coefficient of digestibility of nitrogen are striking in being almost identical for the two subjects when on A. W.'s diet, thus illustrating similarity of digestion and the assimilation process in different individuals. The same thing was seen with three of the five adult subjects in J. W.'s experiment (1), the coefficients for the two persons assimilating the same percentage of nitrogen as did J. W., being 87.7 and 86.1 respectively. The remaining two subjects had somewhat lower nitrogen coefficients, but agreed with each other, 82.5 and 83.1.

Although the fiber content of neither diet was determined by analysis or calculation (figures for the fiber content of cooked apple, and canned peaches have not been found in the literature), it is thought that J. W.'s diet contained a greater amount of indigestible fiber than did A. W.'s. According to Bulletin No. 28 and Circular No. 50, U. S. Department of Agriculture, celery contains 0.98 per cent fiber and lettuce but 0.7 per cent. The figures for raw apples are 1.0 per cent fiber, and for raw peaches 0.6 per cent. It is true that the difference in the amount of celery eaten by J. W. on her own diet (170 grams) as compared with lettuce in A. W.'s diet (100 grams) is the same as the difference in consumption of lettuce by J. W. and A. W. on the latter's diet (100 grams *vs.* 30 grams); but the actual amount of cellulose would be proportionately higher in the celery and the coarser vascular bundles of celery suggest a greater resistance to digestion than do the more uniformly tender leaves of lettuce. One third (100 grams) of the pared apples in J. W.'s diet was eaten raw, the remaining 200 grams baked; the entire 300 grams of peaches in A. W.'s diet was the canned fruit.

TABLE II  
UTILIZATION OF NITROGEN AND FAT

Subject			Diet			Period			Nitrogen					Fat						
									Intake per day	Outgo—Av. Daily			Balance	Absorbed	Coeffi- cient	Intake per day	Outgo in feces		Balance	Coeffi- cient
										In Urine	In Feces	Total					3-days	Av. daily		
.W.	J.W.'s	1	Gms. 11.05	Gms. 9.77	Gms. 1.65	Gms. 11.42	Gms. -0.37	Gms. 9.40	85.1	Gms. 93.6	Gms. 4.90	Gms. 1.63	Gms. 92.0	98.2						
		2	11.30	9.26	1.45	10.71	+0.59	9.85	87.2	99.0	7.63	2.54	96.5	97.4						
		3	11.41	9.85	1.50	11.35	+0.06	9.91	86.9	95.5	7.49	2.50	93.0	97.4						
	A.W.'s	1	8.88	7.89	0.99	8.88	0.00	7.90	88.8					Not deter- mined						
		2	9.17	8.00	0.99	8.99	+0.18	8.18	89.0											
	A.W.	A.W.'s	1	8.53	7.88	0.96	8.84	-0.31	7.58	88.8					Not deter- mined					
2			9.00	7.53	1.08	8.61	+0.39	7.92	89.0											



With respect to the influence of cooking upon cellulose, Woodruff (20) has recently reported evidence in experiments with rats "that cooking of potato cellulose diminished the amount of it which could be recovered in the feces, no matter whether the cellulose was fed as potato itself or as fiber added to the basal ration. When a raw basal ration with raw potato fiber was fed, 58 per cent of the cellulose fed was recovered in the feces. Cooked potato fiber with the basal ration allowed for only a 31 per cent recovery. The raw potato diet showed a recovery of 83 per cent of the fiber, whereas cooked potato showed only 60 per cent." On this evidence, then, the greater amount of raw fiber in J. W.'s diet might reasonably be expected to interfere more with digestion than did the cooked fiber of A. W.'s diet.

While both diets in the Chicago studies were entirely satisfactory to maintain the habitual rhythm of bowel evacuation in the subjects, the effect of the greater bulk in J. W.'s diet seems to be shown in the slightly but consistently lower coefficient of digestibility for nitrogen in J. W. when on her own diet, than when on A. W.'s diet (85.0, 87.0, 86.9 *vs.* 88.8 and 89.0), and more clearly in the 50 per cent higher fecal nitrogen of J. W., 1.65, 1.45, 1.50 gms. on her own diet, as contrasted with 0.99 and 0.99 on A. W.'s diet.

An interesting contrast to the effect of a liberal amount of fiber upon the utilization of protein, is shown by the fact that the coefficients of digestibility of fat for J. W. upon her own diet, 97.4, 98.2, 97.4, are slightly higher than the usually accepted figure, 95.

With these observations, McLaughlin's (14) findings are in accord, for when relatively large amounts of spinach, 250 to 325 grams per day,\* replaced milk as a source of calcium in her experimental diet, the average coefficient for protein for seven subjects fell from 91.9 on the milk-containing diet to 88.6 on the spinach-containing diet. One subject in her experiments had the same nitrogen coefficient on the spinach-containing diet as on the milk-containing diet. When the figures for the remaining six subjects are averaged, the nitrogen coefficient on the spinach-containing diet becomes 87.4, and the milk-containing diet 92.8. The average coefficients for fat in McLaughlin's experiment are the same on the two diets, 97.2 and 97.7, and are slightly higher than the accepted average coefficient for fat.

Another interesting similarity between McLaughlin's study and these done in the University of Chicago is seen in the water content of the feces. The following percentages of water are reported: for McLaughlin's

---

\* Thanks are here expressed to Dr. McLaughlin for use of data from her private files.

two periods 80 and 82 respectively; for J. W. on her own diet 80; for J. W. on A. W.'s diet 80.5; and for A. W. on her own diet 80.6.

Woods and Merrill (3) in their tests of digestibility of white, entire wheat, and Graham bread when each was eaten with milk, found these coefficients:

	<i>Protein</i>	<i>Fat</i>
Milk and white bread	93.6	95.5
Milk and entire wheat bread	91.7	96.5
Milk and Graham bread	88.6	95.5

While the percentage of available nitrogen decreases with the increase of fiber in the diet, the availability of the fat remains practically the same. Blunt and Mallon (21) in their study of the digestibility of bacon used an average amount of 119 grams of much-cooked or 158 grams of slightly-cooked bacon, together with large amounts of shredded wheat (390 to 490 grams) and 1080 cc. to 1450 cc. of orange juice and a little sugar as a daily diet. They found the average coefficient for fat to be 92.8 when the bacon was slightly cooked, and 95 when much cooked, thus agreeing with the usually reported figures. The corresponding average nitrogen coefficients were 79.8 and 80.0. These authors comment: "The nitrogen of the diet was not so well utilized. These low figures, however, are undoubtedly due not to the bacon nitrogen but to the shredded wheat the coefficient of digestibility of which has been reported to be 57.7." Similarly, Holmes' (10) study of the digestibility of wheat bran, when eaten along with potato, apple, orange, butter and sugar, showed an average coefficient of 93.1 for the fat of the entire diet, whereas the coefficient for the protein was very low, being but 45.3, when the cases of negative nitrogen balance are excluded in calculating the averages. The uniformly high coefficients found for both vegetable and animal fats (with two exceptions 95 or above), in the extensive series of tests made in the Department of Agriculture, also testify to the non-interference of the fiber (from wheat biscuit, apple and orange) with the utilization of fats in a mixed diet.

These results in human studies corroborate Mitchell's (22) findings upon the effect of roughage on the fecal nitrogen of rats. He fed four litter mate rats a nitrogen-free diet. In one test the rats were allowed to eat filter paper in addition to the diet. With paper in the diet the fecal nitrogen was 180, 164, 159, 191 mg.; without paper 124, 111, 130, 123 mg. respectively for the four rats. The average of 173 mg. fecal nitrogen excreted when filter paper was consumed, is an increase of 42 per cent over the average of 122 mg. fecal nitrogen on a nitrogen-and-fiber-free diet.

The lower-than-average coefficient for nitrogen in diets which are high

in fiber may have two possible explanations, singly or in union. The large amount of fiber may interfere with the action of proteolytic enzymes; the diet, as a whole, may occasion an unusually large outgo of metabolic nitrogen.

#### SUMMARY

It may be concluded on the basis of the data from the experiments which are drawn upon for this report, that a high proportion of bulk in the diet tends to reduce the utilization of nitrogen to a lower plane, but that it does not have the same effect upon the utilization of fat in the diet.

#### BIBLIOGRAPHY

1. Whitacre, J., and Blunt, K., Coefficient of digestibility and dynamic action of a simple diet in contrasting types of individuals. *Jour. Home Econ.*, 1927, XIX, 1.
2. Willard, A., and Blunt, K., A comparison of evaporated with pasteurized milk as a source of calcium, phosphorus, and nitrogen. *Jour. Biol. Chem.*, 1927 LXXV, 251.
3. Woods, C. D., and Merrill, L. H., A report of investigations on the digestibility and nutritive value of bread, *U. S. Dept. of Agr. Off. Exp. Stations Bull.* 85, 1900.
4. Doane, C. F., The digestibility of cheese. *U. S. Dept. of Agr. Bureau of Animal Industry, Circular* 166, 1911.
5. Langworthy, C. F., and Holmes, A. D., Digestibility of some animal fats. *U. S. Dept. of Agr. Bull.* 310, 1915.
6. Langworthy, C. F., and Holmes, A. D., Digestibility of grain sorghums. *U. S. Dept. of Agr. Bull.* 470, 1916.
7. Langworthy, C. F., and Holmes, A. D., Digestibility of very young veal. *Jour. Agr. Research*, 1916, VI, 577.
8. Langworthy, C. F., and Holmes, A. D., Digestibility of some vegetable fats. *U. S. Dept. of Agr. Bull.* 505, 1917.
9. Holmes, A. D., Studies on the digestibility of some nut oils. *U. S. Dept. of Agr. Bull.* 630, 1918.
10. Holmes, A. D., Experiments on the digestibility of wheat bran in a diet without wheat flour. *U. S. Dept. of Agr. Bull.* 751.
11. Langworthy, C. F., and Deuel, H. J., Jr., Digestibility of raw corn, potato, and wheat starches. *Jour. Biol. Chem.* 1920, XLII, 27.
12. Rose, M. S., and Macleod, G., Some human digestion experiments with raw white of egg. *Jour. Biol. Chem.* 1922, L, 83.
13. Rose, M. S., and Macleod, G., Digestion experiments with the raw white of egg. II. The digestibility of unbeaten in comparison with beaten whites. *Jour. Biol. Chem.*, 1923, LVIII, 369.
14. McLaughlin, L., Utilization of the calcium of spinach. *Jour. Biol. Chem.* 1927, LXXIV, 455.
15. Sherman, H. C. Chemistry of food and nutrition. N. Y. 3d ed., 1926.
16. Koch, F. C., and McMeekin, T. L., A new direct nesslerization micro-Kjeldahl method and a modification of the Nessler-Folin reagent for ammonia. *Jour. Amer. Chem. Soc.* 1924, XLVI, 2066.
17. Chaney, M. S. and Blunt, K., The effect of orange juice on the calcium, phosphorus, magnesium, and nitrogen retention and urinary organic acids of growing children. *Jour. Biol. Chem.*, 1925, LXVI, 829.

- 
18. Official and tentative methods of analysis of the Ass'n of Official Agricultural Chemists, Washington, 1925.
  19. Jephcott, H., Estimation of fat, lactose, and moisture in dried milks. *Analyst*, 1923, XLVIII, 529.
  20. Woodruff, S. and Miller, E., Studies upon edible cellulose I. Recovery of crude fiber from raw and cooked potato cellulose. *Jour. Home Econ.* 1928, XX, 754.
  21. Blunt, K., and Mallon, M. G., Digestibility of bacon. *Jour. Biol. Chem.*, 1919, XXXVIII, 43.
  22. Mitchell, H. H., A method of determining the biological value of protein. *Jour. Biol. Chem.* 1923, LVIII, 873.



---

# THE NUTRITIVE VALUE OF NEW ZEALAND SPINACH

BY LAURA McLAUGHLIN

(From the Foods and Nutrition Department, with the Cooperation of the Department of Vegetable Crops, Iowa State College, Ames.)\*

Received for Publication—July 3, 1929

NEW ZEALAND spinach (*Tetragonia expansa*) is a comparatively new kind of market greens. Its particular advantage is in its being heat tolerant. In the hot summer months when spinach and some other green vegetables cannot be grown, New Zealand spinach, which is not a variety of spinach (*Spinacia oleracea*), is easily grown as a truck crop and furnishes succulent greens for months. Furthermore it is being grown as a winter crop in some eastern greenhouses. The tender, rather fleshy leaves have a distinct flavor more pleasing to some than that of common, mild spinach.

The nutritive value of any vegetable used as a salad or for greens is dependent largely on its mineral and its vitamin contents. The few references to New Zealand spinach in the literature (3, 7) show it to be high in minerals, especially iron. No references were found to its vitamin content. Its appearance, flavor and composition, as found in our experimental work, are recommendation enough. It is compared with spinach only because this has previously been shown to be exceptionally rich in minerals and potent in vitamins. The use of spinach has been advocated to such an extent that recognition of other vegetables with different flavors is needed before unfavorable reaction towards eating spinach becomes too great.

## MINERAL CONTENT

Raw and cooked samples were analyzed for moisture, total ash, calcium, phosphorus and iron. Leaves alone were used for analysis because much of the stem, particularly in older plants, is tough and fibrous. Samples of New Zealand spinach, as gathered in the college greenhouse or garden, showed no dirt visible to the eye and washing yielded a negligible amount of foreign matter. Therefore, the raw vegetable was analyzed without washing. It was weighed as soon as possible after picking and put into a vacuum oven,

---

\* Spinach and New Zealand spinach were grown and furnished through the courtesy of Dr. E. S. Haber. Most of the total ash, calcium and phosphorus determinations were made by Carrie Hodges, some of the vitamin A determinations by Lena Gilbert and some of the vitamin B determinations by Iva Mullen, in partial fulfillment of the requirements of each student for the degree of Master of Science.

set at 70° C, to get the moisture content. The dried material was ashed in an electric muffle furnace at dull red heat. The ash for calcium and phosphorus determinations was dissolved in hydrochloric acid as described by Clark (2). Calcium was precipitated as the oxalate in the presence of acid (pH 4.8) and determined by titration against permanganate (9). Phosphorus was determined by a modification of the Pemberton-Kilgore method (6), precipitating the phosphomolybdate, dissolving this in standard sodium hydroxide, and back titrating. Iron was determined colorimetrically by the Walker method (4).

As shown in Table I, raw New Zealand spinach is exceptionally high in mineral salts, is a very good source of iron and of calcium but not of phos-

TABLE I  
PERCENTAGE COMPOSITION OF NEW ZEALAND SPINACH LEAVES<sup>1</sup>

	Water	Water	Ash	Ash	Ca	P	Fe	Protein (Nx6.25)	Oxalic acid, anhydrous
Av.	94.0 <sup>1</sup>	88.4 <sup>2</sup>	1.92 <sup>1</sup>	2.43 <sup>2</sup>	.045	.027	.0023	1.4	.49
P.E. <sup>3</sup>	0.3	1.1	0.01	0.05	.002	.002	—	0.2	.03
Max.	95.0	89.8	1.94	2.58	.050	.030	.0024	1.7	.53
Min.	93.3	85.6	1.90	2.38	.038	.022	.0022	1.1	.40
No. anal.	18	11	5	6	10	6	5	8	8

<sup>1</sup> Samples grown in greenhouse.

<sup>2</sup> Samples grown in garden.

<sup>3</sup> Probable error of the mean.

<sup>4</sup> Lichtin (7) gives the following percentages: 1.86 ash, and 0.00456 iron, in New Zealand spinach grown at Cornell; 2.59 ash, and 0.00328 iron, in samples from the open market.

phorus. These facts are brought out by comparison with other common leafy vegetables shown in Table II.

Changes in mineral content caused by cooking are brought out in Table III. The greens were prepared for cooking in the usual way and two methods of cooking were employed. First, the weighed leaves were washed in tap water and cooked in the clinging wash water only in the top of an enamel double boiler; second, the weighed sample, after washing, was put into a measured volume of boiling tap water and cooked for a definite period. In each case the cooked product was drained and both the spinach and the cooking liquor dried and analyzed. Mineral contents were calculated on the basis of the fresh raw weight.

New Zealand spinach cooked without added water retains its distinctive

flavor with a somewhat astringent after taste. Fifty minutes in a double boiler gave a mushy product, thirty-five minutes a tender one though the brilliancy of the green color was lost. One-sixth of the mineral salts, including one-eighth to more than one-fourth of the iron, was drained off

TABLE II  
PERCENTAGE COMPOSITION OF SOME LEAFY VEGETABLES

	Water <sup>1</sup>	Ash	Ca <sup>2</sup>	P <sup>2</sup>	Fe	Oxalic acid, anhydrous
Cabbage	91.5	1.0 <sup>1</sup>	.045	.029	.0011 <sup>3</sup>	.0056 <sup>4</sup>
Lettuce	94.7	0.9 <sup>1</sup>	.043	.042	.0007 <sup>3</sup>	trace <sup>4</sup>
Spinach	92.3	2.1 <sup>1</sup> 1.41 <sup>2</sup>	.067	.068	.0036 <sup>3</sup> .0061 <sup>3</sup> .0031 <sup>4</sup>	.299 <sup>4</sup>

<sup>1</sup> Atwater and Bryant (1).

<sup>2</sup> Lichtin (7).

<sup>3</sup> Sherman (11, appendix B).

<sup>4</sup> Spinach grown under same conditions as New Zealand spinach of our analyses.

<sup>5</sup> Floyd, L. P., Unpublished Master's Thesis, Univ. of Chicago, 1923.

<sup>6</sup> Arbenz, E., *Mitt. Lebensm. Hyg.* 1917, VIII, 98.

TABLE III  
COOKED NEW ZEALAND SPINACH LEAVES

	Wt. of raw sample	Vol. of water	Time cooked	Green color	Flavor	Percent Mineral Losses calculated on basis of 100 g. raw			
						Ash	Ca	P	Fe <sup>2</sup>
A <sup>1</sup>	gr. 150	cc. *	min. 50	olive	very strong	28.4	5.1	—	
B	150	*	35	"	Strong	15.6	4.5	—	
C	420	*	35	"	"	14.8	4.7	5.8	13
D	420	*	35	"	"	15.6	4.5	5.5	28
E	420	*	35	"	"	15.7	5.1	5.8	28
A	150	500	20	bright	Mild	44.8	6.1	—	
B	150	150	14	"	"	41.9	6.1	—	
C	140	750	10	"	"	39.8	6.1	63.4	37
D	420	750	10	"	"	40.6	5.9	62.9	40
E	420	750	10	"	"	40.5	6.8	62.4	44
F	420	750	10	"	"				47

\* Water clinging to leaves from washing.

<sup>1</sup> Samples A, B and D were drained 10 min., C, 60 min.; and E, 5 min.

<sup>2</sup> Iron was not determined in the same samples as those used for analyses of other minerals. All samples for iron determinations were drained for 10 min.



in the strong cooking liquor but little of the calcium or phosphorus was lost.

Ten minutes cooking in excess boiling tap water gave an attractive tender product with a very mild flavor. Draining away the liquid took with it little calcium but about 40 per cent of the total mineral salts and of the iron, and more than 60 per cent of what phosphorus the raw vegetable contained.

Using our figures for composition of New Zealand spinach, and average composition figures of Atwater and Bryant (1) and of Sherman (11) for the other common leafy vegetables, New Zealand spinach, even when cooked in a large volume of water and drained longer than is customary before serving, is found to be as rich in mineral salts as raw spinach and to be richer in iron and as high in calcium as lettuce or cabbage served raw.

#### VITAMIN POTENCY

Only raw leaves were tested for vitamin content. Albino rats from our stock colony were used for most determinations. The diet of the mothers of other rats used had been similar, that is, largely grain with milk added. Our stock diet is Steenbock's (14) modified to contain wheat germ and yeast, and supplemented at intervals by lettuce. The laboratory technic was that of Ferry (5). Rats were weaned at 28 or 29 days and placed directly on the experimental diet in individual cages. The basal diet consisted of purified casein 18 per cent, dextrinized cornstarch (8) 76 per cent, salt mixture (10) 4 per cent, and agar 2 per cent.

*Vitamin A.* For vitamin A tests the casein was made A-free (12), the diet irradiated and a salt spoonful of yeast, weighing approximately 0.4 gram, was fed separately six times a week. The method used was essentially that of Sherman and Munsell (12). Cessation of growth was the chief criterion for deciding when the store of vitamin was depleted. The basal diet was then supplemented during the 8-week period with carefully weighed quantities of New Zealand spinach or spinach leaves (Bloomsdale) having, so far as possible, an upper surface area of 4 to 5 square inches. To minimize errors in weighing, the very small quantities of leafy tissue were fed three times a week. Twenty-six negative controls, with an average weight of 50 grams at 28 days, survived 53.5 days on the average or 21 to 22 days after the average depletion period.

Close to 90 mg. of New Zealand spinach per week were required to allow a gain in weight of approximately 3 grams a week for 8 weeks, after depleting our rats which at 28 days weighed on the average about 50 grams. A slightly smaller quantity of ordinary spinach grown under the same

conditions induced a somewhat larger gain with a correspondingly greater consumption of the basal diet. However, it is clearly shown that New Zealand spinach, like spinach, is several times as rich in vitamin A as are most green foods that have been tested.

*Vitamin B.* The method used was essentially that of Sherman and Spohn (13). Vitamins B and G were considered as an entity. The same

TABLE IV  
VITAMIN A TESTS

New Zealand Spinach							
Amt. per wk.	No. of rats	Wt. at 28 days.	Pre-period	Initial wt.	Gain in period	Food eaten weekly	Rats surviving period
gr.		gr.	days	gr.	gr.	gr.	no.
0.09	16	52	33	127	27.4	66	11
0.08	15	50	30	109	16.8	52	12
0.07	15	49	35	119	-11.6	54	8
Spinach							
0.07	7	46	30	125	32.5	69	7

TABLE V  
VITAMIN B CONTENT OF LEAVES

New Zealand Spinach							
Amt. per day	No. of rats	Initial wt.	Weekly change in wt.	Food eaten weekly	Time of survival	Rats surviving period	Gain in wt. of survivors
gr.		gr.	gr.	gr.	days	no.	gr.
1.0	7	50	-1.5	24.6	41	1	8
1.2	7	47	-0.4	25.6	49	4	2
1.4	8	50	-0.3	27.3	49	4	10
1.6	8	37	+3.3	21.4	56 (K)	8	26
Spinach							
1.0	8	49	+2.5	32.6	50	6	29
1.2	10	49	+3.4	34.9	56 (K)	10	27
1.4	7	37	+6.5	35.8	56 (K)	7	52

K denotes killed.

basal diet was used as for vitamin A determinations except that it was not irradiated and the casein was made B-free (13). Cod-liver oil, five drops per day, was fed separately. The New Zealand spinach and the spinach was the same as for vitamin A tests but was fed six times per week with a double portion on Saturdays. Fifteen rats, with an average weight of 46 grams at 28 days, used as negative controls, survived for 40 days on the average, with an average loss of 2.6 grams in weight per week.

It requires a little more than 1.4 grams of New Zealand spinach daily to maintain a 50 gram rat at constant weight for 8 weeks. On 1.6 grams daily, 37 gram rats make a distinct gain, but just one-half as great as that made by rats of the same weight getting 1.4 grams of spinach (*Spinacia oleracea*). The latter rats showed an appetite for the basal diet one-half again as great. New Zealand spinach, though it contains somewhat less vitamin B complex than does spinach, belongs in the same class with it.

#### SUMMARY

New Zealand spinach is especially valuable in the diet because of its high salt content. It compares favorably with most green vegetables in iron and calcium.

Cooked without addition of water, it retains most of its minerals.

If greens of milder flavor and brighter color are preferred, it may be boiled in water with little loss of calcium but with much loss of other minerals. However, it still retains enough to compare favorably with raw cabbage or lettuce.

New Zealand spinach is exceedingly rich in vitamin A and is a good source of vitamin B complex.

#### REFERENCES

1. Atwater, W. O. and Bryant, A. P., *U. S. Dept. Agr., Office Exp. Sta.*, Bul. 28, 1906.
2. Clark, G. W., *Jour. Biol. Chem.*, 1925, LXV, 597.
3. Courtney, A. M., Fales, H. F. and Bartlett, F. H., *Amer. Jour. Dis. Child.* 1917, XIV, 34.
4. Elvehjem, C. A. and Hart, E. B., *Jour. Biol. Chem.*, 1926, LXVII, 43.
5. Ferry, E. L., *Jour. Lab. Clin. Med.* 1920, V, 735.
6. Hibbard, P. L., *Jour. Ind. Eng. Chem.* 1913, V, 988.
7. Lichtin, A., *Amer. Jour. Phar.*, 1924, XCVI, 361.
8. McCollum, E. V., and Davis, M., *Jour. Biol. Chem.* 1915, XX, 641.
9. McCrudden, F. H., *Jour. Biol. Chem.*, 1911, X, 187.
10. Osborne, T. B. and Mendel, L. B., *Jour. Biol. Chem.* 1919, XXVII, 557.
11. Sherman, H. C., *Chemistry of Food and Nutrition*, Third Ed., 1927, New York.
12. Sherman, H. C. and Munsell, H. E., *Jour. Amer. Chem. Soc.* 1925, XLVII, 1639.
13. Sherman, H. C. and Spohn, A., *Jour. Amer. Chem. Soc.*, 1923, XLV, 2719.
14. Steenbock, H., *Science* 1923, LVIII, 449.

---

## CYCLIC VARIATIONS IN THE BASAL METABOLIC RATE OF WOMEN

BY F. A. HITCHCOCK AND FRANCES R. WARDWELL

(*From the Department of Physiology, Ohio State University, Columbus.*)

Received for Publication—July 5, 1929

THE various workers who have from time to time reported experiments dealing directly or indirectly with the effect of the menstrual cycle on the metabolic rate of women have often differed profoundly in their conclusions. Among the chief investigators who have concluded that there was no demonstrable effect may be listed Zuntz (1906), Gephart and DuBois (1916), Blunt and Dye (1921), Wiltshire (1921), and Lanz (1924). Gephart and DuBois deal with the subject only indirectly. They report three tests on a single female subject so that it is not at all surprising that they failed to show an effect. Blunt and Dye's fourteen subjects actually averaged 1.6 per cent lower in their metabolic rate during menstruation than at other times but these investigators point out that variations of this magnitude may occur at any time without apparent cause.

The one point upon which there seems to be the most general agreement is the existence of the so-called premenstrual rise. An increased metabolic rate which occurred sometime during the week preceding the onset of menstruation has been emphasized by Snell, Ford and Rowntree, (1920). These investigators found that the rise in the metabolic rate often occurred during the catamenia itself. Other investigators who have noted a similar rise in the metabolic rate of their female subjects are Rowe and Eakin (1921) and Wakeham (1923).

A decrease in the metabolic rate occurring on the first or second day of menstruation has been noted by Kunde (1923), Hafkesbring and Collett (1924), and Collett and Liljestrand (1924). Collett and Liljestrand found that the metabolic rate reached a minimum on the first or second day of menstruation and that there was a second low point ten to eighteen days later.

Quite recently Rogers and Flemming (1928) have reported experiments on seven normal women from which they conclude that there is "a relation between the metabolic rate and the menstrual cycle with the lowest point in the metabolic cycle coming during or immediately following the menstrual period." Another recent paper is that of Benedict and Finn

(1928). They have published valuable and interesting data on the gaseous metabolism of one female subject over a period of years. With this subject the average oxygen consumption during menstruation was 3 per cent lower than for the inter-menstrual periods. In evaluating their data the authors emphasize the unusually good physical condition of this subject. Still more recently Griffith (1929) and his coworkers have published data which include observations on the basal metabolism of three women over a period of one year. All three of these women showed a cyclic variation in the metabolic rate which was synchronous with the menstrual cycle. However, the individual cycles were not identical. One subject showed the lowest rate during menstruation with a progressive rise during the intermenstrual period. The remaining two subjects showed the lowest rate in the second or third week of the menstrual cycle. All three, however, showed an increased metabolic rate during the third or fourth week of the cycle.

When one considers this mass of conflicting evidence it appears that the only hope of reaching satisfactory conclusions lies in the accumulation of sufficient data to justify statistical treatment. With this idea in mind we began, some three years ago, an investigation of the day-by-day variations in the basal metabolism of women. The apparatus used was the Benedict-Roth-Collins apparatus with kymograph attached. All calculations were made from the graphic record of the respirations of the subject. In general the technic was that described and recommended by Roth (1923). During the course of the investigation more than eight hundred basal metabolism determinations were made on twenty-seven women. In the final analysis of data only those subjects who were under observation for at least two complete menstrual cycles were included. This rule resulted in the elimination of six subjects. One additional subject was ruled out on account of her rather erratic conduct. It was felt that she was not cooperating as fully as could be desired and that the tests made on her were, therefore, not altogether reliable. This left twenty subjects in the final compilation of data, upon whom a total of 625 basal metabolism determinations was made. Of these, 155 tests were made during menstrual periods; the remaining 470 between menstrual periods. All tests were run in duplicate and the lower one used. This was done on the supposition that many things might happen to a subject that would increase her metabolic rate but that aside from sleep nothing could happen that would decrease it. In no case did any of the subjects fall asleep during a test. The average difference between the duplicate tests was less than three per cent.

It was usually found that the tests run on the first day any particular subject came to the laboratory gave results appreciably higher than those obtained in subsequent tests. Therefore, tests made on the first day were in every case discarded. In a few cases it was found necessary to discard the test made on the first two or three days since they were well above the metabolic rate which subsequent tests showed to be normal for the subject. This observation would indicate that it is rarely, if ever, safe to base scientific or clinical findings on a single basal metabolism test. This point has also been emphasized by Boothby and Sandiford (1920).

The body temperature (oral), the pulse rate, and both systolic and diastolic blood pressure were determined at each test. This was done toward the end of the rest period after the subject had been lying down at least fifteen minutes. Tests were run on each subject two or three times a week. During the last three months of the experiment four of the most reliable of the subjects were selected and tests were run on each of these daily except Sunday. During this period two male subjects were run daily, the procedure for them being the same as that already described for the women. A graph was prepared for each subject upon which the metabolic rate, pulse rate, body temperature, and blood pressure were all shown. A close inspection of these graphs usually indicated a direct correlation between pulse rate and metabolic rate, but this correlation was by no means perfect. No relationship between the metabolic rate and any of the other measurements made was apparent. A careful study of the correlations between these various physical measurements is being made and it is our intention to make it the subject of a future paper.

At the end of the experiment the tests made on each subject during menstrual periods were grouped together and the arithmetical mean computed. The standard deviation and the probable error of the mean were then determined. All tests that had been made between menstrual periods on the same subject were then placed in a second group and subjected to the same mathematical treatment. The difference between the arithmetical mean of the menstrual tests and the non-menstrual tests was then determined and the probable error of this difference was computed by taking the square root of the sum of the squares of the probable errors of the two means. The difference between the two means was then divided by the probable error of the difference and this number used as an index of the significance of the deviation between the average of the menstrual tests and that of the non-menstrual tests. These statistical data are shown in Table I.

It will be noted from this table that fourteen of the twenty subjects showed a lowering of the metabolic rate during the menstrual period. In one subject the average metabolic rate during the menstrual periods was exactly the same as the average rate between menstrual periods and the remaining five subjects showed a slight increase in the metabolic rate during menstruation. The subjects showing decreased metabolic rate during menstruation have been arranged according to the decreasing

TABLE I

COMPILATION OF STATISTICAL DATA SHOWING THE TENDENCY FOR THE BASAL METABOLIC RATE OF WOMEN TO BE LOWERED DURING THE MENSTRUAL PERIOD

Subject	Intermenstrual period			Menstrual period			Diff.	P.E. of diff.	Diff. divided by P.E.
	No. of tests	Avg. Cal. per sq. m. per hr.	Stand. dev.	No. of tests	Avg. Cal. per sq. m. per hr.	Stand. dev.			
A.C.	14	34.0	1.11	2	31.3	0.40	-2.7	0.28	9.7
M.P.	12	39.9	2.17	2	36.7	1.20	-3.2	0.71	4.5
L.S.	13	35.5	3.74	4	32.1	0.89	-3.4	0.83	4.1
S.R.	52	36.3	1.83	13	34.9	1.70	-1.4	0.36	3.9
R.D.	12	36.5	1.76	3	35.1	0.33	-1.4	0.37	3.7
F.R.	91	33.3	1.89	54	32.6	1.66	-0.7	0.20	3.5
D.W.	11	41.6	2.45	3	39.1	2.05	-2.5	0.94	2.7
A.B.	78	35.8	3.20	17	34.9	2.14	-0.9	0.43	2.1
E.C.	18	36.3	2.79	4	34.7	2.08	-1.6	0.83	1.93
C.B.	13	35.6	2.82	3	34.0	1.72	-1.6	0.85	1.88
N.R.	8	38.4	1.61	2	36.4	3.25	-2.0	1.58	1.26
M.K.	19	40.8	2.38	6	40.5	1.43	-0.3	0.54	0.55
I.K.	10	38.6	1.55	4	38.1	3.01	-0.5	1.06	0.47
R.P.	40	33.8	1.35	19	33.7	1.54	-0.1	0.28	0.36
B.P.	11	37.1	0.72	2	37.1	0.15	0.0	0.16	—
R.S.	13	33.3	2.01	2	33.7	0.10	+0.4	0.38	1.05
G.J.	12	35.8	3.01	2	36.6	0.90	+0.8	0.73	1.11
M.B.	12	33.8	1.19	4	34.9	1.37	+1.1	0.87	1.26
M.S.	13	34.2	3.04	3	37.2	4.13	+3.0	1.71	1.75
M.C.	18	38.4	2.63	6	39.6	1.66	+1.2	0.62	1.94

value of the quotient obtained when the difference between the two means is divided by the probable error of this difference, while those showing an increased metabolism during menstruation have been arranged according to the increasing value of this quotient. At the top of the table comes A. C. Her metabolic rate during menstrual periods was 2.7 Calories per hour per square meter of body surface less than at other times. The probable error of this difference was only 0.28 Calories; therefore, the quotient

obtained when the difference is divided by its probable error is 9.7. According to the law of probability a difference as much greater than its probable error as this one is would occur fortuitously only once in several hundred billion cases. The second subject, M. P., had a metabolic rate during menstrual periods which averages 3.2 Calories less than her metabolism at other times. This difference is 4.5 times as great as its probable error. A deviation as much greater than its probable error as this would occur by chance only once in about 415 cases. Now it happens that the results obtained on these two subjects might be questioned, since in each case there were only two tests made during menstrual periods and in both cases these two tests were made during the same menstrual period. As previously stated, the tests on all subjects extended over at least two complete menstrual cycles. However, in the case of these two subjects the first test was made near the end of a menstrual period. As already explained, our regular procedure was to omit this first test. Since the experiment was stopped with these two subjects just before the beginning of the menstrual period at the end of the second cycle, this leaves only the one menstrual period for consideration in the final data. However, even if we should omit these two subjects the data would be only slightly less significant. Statistically, any deviation more than 1.8 times its probable error is usually considered significant. Ten of the twenty subjects show minus deviations occurring during the menstrual periods which are more than 1.8 times their probable errors. If we omit the first two, which might be questioned although we believe them to be perfectly valid, we find that according to the laws of chance, negative deviations in the metabolic rate during menstruation would occur fortuitously in the number and size noted only in some 450 cases instead of occurring, as they do in our data, in 18 cases. This means that these deviations are some twenty-five times more numerous than could be accounted for by chance.

On the other hand, of the five cases which show a positive deviation of the metabolic rate during menstruation, there is only one in which the deviation is more than 1.8 times its probable error. Positive deviations of this size and number would occur fortuitously in ten cases. It appears, therefore, that positive variations occur in our data only about half as frequently as could be accounted for on the basis of chance, while negative variations occur some 25 times more frequently than could be expected were they entirely fortuitous. If we include subject M. P., this number would be nearly doubled while the inclusion of A. C. would send it up into the millions. This seems to be excellent proof of the existence of a



tendency for the metabolic rate of women to be lowered during the menstrual period.

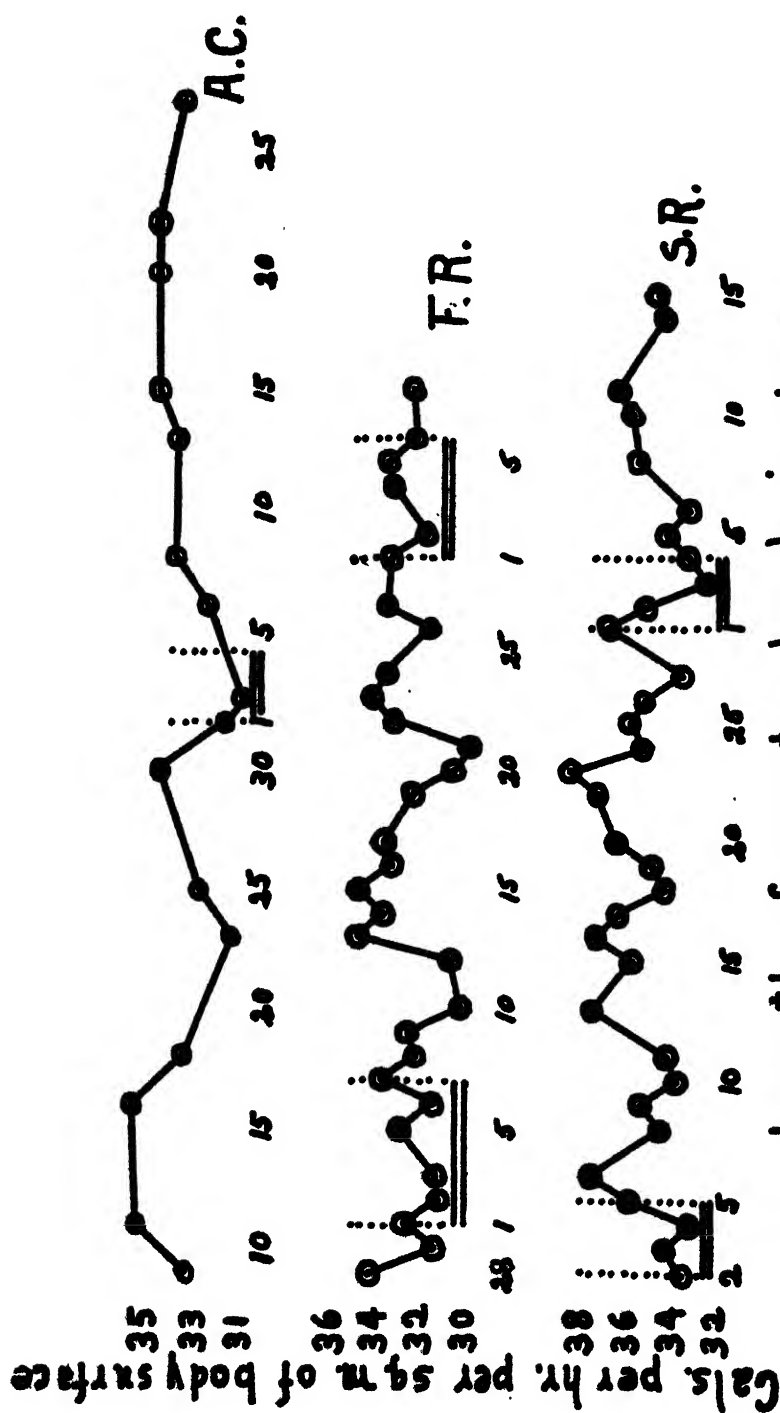
This lowering of the metabolic rate is of slight extent. The average for the ten subjects that showed statistically significant deviations is 5.3 per cent while if all fourteen subjects who show lowered metabolic rates during menstruation are included, the decrease amounts to 4.3 per cent. It would not be difficult to cover up a variation of this magnitude by some uncontrolled factor which acted in the opposite direction. For example Boothby and Sandiford (1924) have suggested pain and discomfort as the true cause of the apparent increase in the metabolic rate of women during menstruation which has been reported several times in the earlier work. It is now universally recognized that this factor must be guarded against. It is altogether probable that there are many other factors, as yet unrecognized, which might cause apparently fortuitous variations in the metabolic rate. The amount of food and exercise taken during the preceding twenty-four hours, the amount of sunlight that has reached the skin, the amount of sleep taken the preceding night or the mental state of the subject might very well produce noticeable changes in the metabolic rate. In this connection we have noted at this laboratory that students who have been at some social function the night preceding the test usually show a lowered metabolic rate. An effect of this same sort was observed during the course of the experiments on M. C. reported in this paper. This young woman was a student in the graduate school of the university. She was under observation for about three months and during this period she took the series of oral and written examinations that are required of all students before they are admitted to candidacy for the degree of Doctor of Philosophy. The average of all tests made on her before she had completed these examinations gave her a metabolic rate of 40.12 Calories per hour per square meter of body surface. After all of her scholastic work had been finished for the school year, her metabolic rate was determined on seven different days scattered over a period of more than four weeks. The average of all of these tests was 35.89 Calories per hour per square meter of body surface, a drop of 10.5 per cent from her previous level. We can suggest no cause for this sudden drop in the metabolic rate of this subject other than mental let-down which naturally accompanied the successful completion of her examinations. We have noted in a number of other cases that the metabolic rates of students were distinctly higher during the week of final examinations than at other times.

F. R. furnishes another example of an effect apparently due to the

mental condition of the subject. At the time these tests were made this subject was finishing her junior year in the College of Medicine. On the evening of June 1, 1928, she was on duty for the first time at the University Dispensary and returned to her room very much excited by this experience. The following morning, the third day of her menstrual period, she had a metabolic rate of 42.35 Calories per hour per square meter of body surface. The average of 54 tests run on this subject during menstrual periods was 32.60 Calories per hour per square meter of body surface, while on the morning of June 1, her metabolic rate had been 31.95 Calories and on June 4 it was again down to 33.23 Calories per hour per square meter of body surface. Here again we can offer no explanation for this very temporary increase in the metabolic rate other than the mental condition of the subject. It is our opinion that in many cases the rather slight change in metabolic rate which accompanies menstruation is concealed by such factors as those enumerated above.

Statistical treatment of our results has been limited to the testing of the significance of the change in the metabolic rate which occurs during the menstrual period itself. Inspection of the curves obtained when the results on individual subjects were plotted shows that this fall in the metabolic rate which accompanied the actual catamenia is not the only cyclic variation in the metabolism to manifest itself. In the great majority of cases the curves show a marked premenstrual rise similar to that emphasized by Rowe and Eakin (1921) and Wakeham (1923). The recently published data of Griffith *et al.* (1929) and of Benedict and Finn (1928) also indicate a premenstrual rise in the metabolic rate. Further discussion of this point, therefore, seems unnecessary. It is sufficient to state that during the week preceding the onset of the menstrual flow our subjects usually showed metabolic rates from five to ten per cent above the average value for the intermenstrual period.

In Figure 1, a portion of the results obtained upon three of the subjects are shown graphically. These curves have been selected because they show most clearly what we have come to believe is the typical cycle through which the metabolic rate of women passes. It can be seen from these curves that the lowered metabolic rate which accompanies the menstrual period often lasts for several days beyond the duration of the menstrual flow. There is then a progressive increase in the metabolic rate which is followed by a second drop which occurs at about the end of the second week of the cycle. This intermenstrual low point is of brief duration, usually lasting for but a day or two. Then comes the premenstrual rise and the drop in the metabolic rate which occurs with the onset of men-  
stru-



Length of menstrual cycle in days.

Figure 1. Graphic representation of a part of the results obtained on three subjects.

ation. It is probable that in some cases where this intermenstrual low point failed to appear, as for example in the second part of the graph for the subject A. C. shown in Figure 1, this was due to the fact that a test was not made on the particular day on which the metabolic rate was at this low level. With most of the subjects it must be remembered that tests were run only two or three times a week.

TABLE II

COMPILATION OF DATA SHOWING THE OCCURRENCE AND MAGNITUDE OF THE INTERMENSTRUAL DROP IN THE METABOLIC RATE

Subject	No. of menstrual cycles studied	Avg. length of cycle in days	No. of intermenstrual drops noted	Day on which intermenstrual drop occurred	Magnitude of intermenstrual drop in per cent	Magnitude of menstrual drop in per cent
A.C.	2	32	1	23	7.0	7.9
M.P.	2	30	1	15	3.8	8.0
L.S.	2	29	1	17	10.0	9.6
S.R.	5	28	5	12	3.1	3.9
R.D.	2	29	2	14	1.6	3.8
F.R.	9	28	5	13	6.8	2.1
D.W.	2	30	0	—	—	4.9
A.B.	9	30	6	13	2.8	2.5
E.C.	2	32	2	13	8.7	4.4
C.B.	2	29	2	19	6.5	4.5
Average	3.7	29.7	2.5	14	5.03	5.16

In Table II, the occurrence of these intermenstrual low points for the ten subjects that showed a significant lowering of the metabolic rate during menstruation has been tabulated. For these ten subjects there was a total of thirty-seven menstrual cycles studied and in twenty-five of these a low point occurred approximately midway between menstrual periods. These low points occurred in nine of the ten subjects included in the table, D. W. being the lone exception. There were only eleven tests made on this subject during the two intermenstrual periods she was under observation, the smallest number made on any subject in this group, and it is therefore altogether possible that the low points occurred on days when no test was made. Likewise, it seems probable that the other subjects would have shown these low points more frequently if the tests had been made at shorter intervals. As it is, they occur in about seventy per cent of the cycles. This is too large a percentage to be accounted for on the basis of chance. In extent they range from 1.6 to 10 per cent below

the average for the intermenstrual periods. The average for the group of ten including D. W. is 5.03 per cent. It is interesting to note that this is the same order of magnitude as the fall which occurs during the menstrual flow. For the same ten subjects the metabolism during menstruation averaged 5.16 per cent lower than during the intermenstrual periods. It is also noticeable from the table that there is a high degree of correlation between these two drops; that is, subjects who show the most pronounced drop in the metabolic rate during menstruation usually show the most marked intermenstrual low point.

The experiments of Benedict and Finn (1928) on Miss W. show similar intermenstrual low points. The thirty-three tests run on her during intermenstrual periods showed an average oxygen consumption of 179.8 cc. per minute. On February 29, the sixteenth day of the menstrual cycle, it dropped down to 176.0 cc. per minute and on March 28, the eighteenth day of the following cycle, it dropped to 175 cc. per minute. These two intermenstrual low points are 2.4 per cent below the average for the intermenstrual period as a whole, while the eleven tests made during menstrual periods average 3.3 per cent lower than the intermenstrual average. It is evident, therefore, that the results of Benedict and Finn as well as our own agree with the earlier work of Collett and Liljestrand (1924) in showing the existence of this intermenstrual low point in the metabolic rate.

It appears to be very well established that ovulation occurs 14 to 18 days after the beginning of menstruation, and since the average for this group of ten subjects shows that this drop in menstruation occurs about 15 days after the beginning of menstruation, while with Miss W. it occurred once on the sixteenth and once on the eighteenth, it seems probable that the two phenomena occur approximately simultaneously. The existence of a causal relationship between the two is a question upon which we can only speculate. There has been published recently some evidence which indicates that the metabolic rate in women may be influenced by the amount of ovarian hormone in the blood stream. McClendon, Burr, and Conklin (1929) in a preliminary report have noted the effect on the metabolic rate of women of injecting varying amounts of the ovarian hormone. In two of their five subjects the metabolic rate rose when small doses were injected and fell with the administration of larger doses. Two subjects showed increased metabolic rates following a single injection of a large dose of the hormone, while the fifth subject who received the largest dose of all showed an unchanged metabolic rate. It is possible that these results are complicated by the different

stages of the oestrus cycle in which the various subjects were at the time of the experiment. We shall await the complete report of this work with considerable interest. However, considering this evidence in connection with our own work, we are tempted to suggest that large amounts of the ovarian hormone in the blood stream cause a decrease, while smaller amounts produce an increase in the metabolic rate of women. It is not unreasonable to suppose that relatively large amounts of the ovarian hormone are set free in the blood stream at menstruation and at ovulation. This condition would then, according to our hypothesis, cause a lowered metabolic rate while the smaller amounts of the hormone present in the blood stream at other times would result in a higher metabolic rate.

The fact that two of our subjects were under observation for more than a year (in one case the summer months being omitted) and two others during the winter and spring, makes it possible to use our data in a consideration of the effects of the seasons on the basal metabolic rate. In Table III, the averages for the seasons during which these four subjects

TABLE III  
DATA SHOWING THE EFFECT OF SEASONS ON THE BASAL METABOLIC RATE

Subject	Spring		Summer		Autumn		Winter		Spring	
	No. of tests	Avg. Cal. per sq. m. per hr.	No. of tests	Avg. Cal. per sq. m. per hr.	No. of tests	Avg. Cal. per sq. m. per hr.	No. of tests	Avg. Cal. per sq. m. per hr.	No. of tests	Avg. Cal. per sq. m. per hr.
F.R.	8	33.80	20	33.13	7	35.72	21	33.52	37	33.00
A.B.	12	37.53			14	36.86	16	34.84	36	35.52
S.R.							13	38.15	39	35.71
R.P.							10	33.03	30	34.16

were under observation are tabulated. In obtaining these figures all non-menstrual tests run during any particular season were averaged. It may be seen from this table that F. R. showed a metabolic rate of 33.80 Calories per hour per square meter of body surface during the spring of 1927. There was a slight decrease in this rate during the summer followed by a sharp rise in the autumn. During the subsequent winter and spring it dropped back to a point slightly lower than that noted at the beginning of the experiment. The outstanding characteristic of the records on this subject is the increased metabolic rate shown during the autumn. It is

interesting to note that during the fifteen months this subject was under observation her only vacation occurred in September 1927, and it is the tests run during the two or three months immediately following this vacation that show the increased metabolic rate. This seems to support the theory recently advanced by Benedict (1928) that the basal metabolism is to some extent a measure of the physical fitness of the individual. Subject A. B. was not available for test during the summer months. Her metabolism was only slightly lower in the fall than it had been during the preceding spring. During the winter, however, there was a marked drop followed by a rise in the following spring. The fact that this subject showed a lower metabolic rate in the spring of 1928 than she had in spring of 1927 again can be correlated with her physical condition. During the spring of 1928 she was in rather poor shape physically and from the 18th of April to the 8th of May was ill with what was diagnosed as influenza. Of the two subjects who were under observation during the winter and spring of 1928 S. R. showed a decrease while R. P. showed an increase in the metabolic rate. The same discrepancy in the point at which the metabolism starts up is seen in the case of F. R. and A. B., the former showing decrease and the latter an increase with the coming of spring. The two subjects whose metabolism was lower in the spring were older women who worked harder and probably took less time for recreation than the two subjects whose metabolism increased with the coming of spring. On the whole, our data seem to agree with those published by Gustafson and Benedict, (1928) who showed that the basal metabolism was usually higher in the summer than in the winter.

#### SUMMARY

A study of the day-to-day variations in the basal metabolism of twenty women justifies the following conclusions:

1. There is a strong tendency for the metabolic rate to be lowered during the menstrual period.
2. A second low point in the metabolic rate occurs about the middle of the intermenstrual period.
3. In many cases the mental state of the subject seems to have a marked effect on the basal metabolic rate.
4. There seems to be a seasonal variation with the lowest metabolic rate occurring in the winter or spring and the highest in the summer or autumn. The time of year at which the metabolic rate begins to increase seems to depend to some extent on the personal habits of the subject.

## BIBLIOGRAPHY

- BENEDICT, F. G., 1928. *Amer. Jour. Physiol.*, LXXXV, 650.
- BENEDICT, F. G., and M. D. FINN. 1928. *Amer. Jour. Physiol.*, LXXXVI, 59.
- BLUNT, K. and M. DYE, 1921. *Jour. Biol. Chem.* XLVII, 69, 73.
- BOOTHBY, W. M. and I. SANDIFORD, 1920. A laboratory manual on the technic of basal metabolic rate determinations. Philadelphia.
- 1924. *Physiol. Reviews*, IV, 69.
- COLLETT, M. E. and G. LILJESTRAND, 1924. *Skand. Arch. f. Physiol.*, XLV, 17.
- GEPHART, F. C. and E. F. DUBOIS, 1916. *Arch. Int. Med.*, XVII, 902.
- GRIFFITH, F. R., G. W. PUCHER, K. A. BROWNELL, J. A. KLEIN, and M. E. CARMER, 1929. *Amer. Jour. Physiol.*, LXXXVII, 602.
- GUSTAFSON, F. L. and F. G. BENEDICT, 1928. *Amer. Jour. Physiol.*, LXXXVI, 43.
- HAFKESBRING, R. and M. E. COLLETT. 1924. *Amer. Jour. Physiol.*, LXX, 73.
- KUNDE, M. M., 1923. *Jour. Metab. Research*, III, 399, 445.
- LANZ, W., 1924. *Zeitschr. f. Geburtsh. Gynak.*, LXXXIX, 133.
- MCCLENDON, J. F., G. BURR, and C. CONKLIN., 1929. *Proc. Soc. Exper. Biol. and Med.* XXVI, 265.
- ROTHER, P., 1923. Modifications of Apparatus and Improved Technic Adaptable to the Benedict Type of Respiration Apparatus. Battle Creek.
- ROWE, A. H. and M. EAKIN, 1921. *Calif. State Jour. Med.* XIX, 320.
- ROGERS, C. G. and J. FLEMMING, 1928. *Proc. Ohio Acad. Sci.* VIII, 196.
- SNELL, A. M., F. FORD and L. G. ROWNTREE, 1920 *Jour. Amer. Med. Assoc.*, LXXV, 515.
- WAKEHAMS, G., 1923. *Jour. Biol. Chem.*, LVI, 555.
- ZUNTZ, L., 1906. *Arch. f. Gynak.*, LXXVIII, 106.





JANUARY, 1930

THE NUTRITIVE VALUE OF CEREAL  
BREAKFAST FOODSIII. THE RATE OF DIGESTION AND ABSORPTION AS  
DETERMINED BY EXPERIMENTS ON RATS

BY H. A. MATTILL AND H. G. SMITH

With the assistance of M. M. Clayton

*(From the Department of Vital Economics, University of Rochester,  
Rochester, N. Y.)*

Received for Publication—July 6, 1929

**I**N CONNECTION with the work on the rate of absorption of cereals in human subjects (1) it seemed desirable to make similar experiments upon another animal. Mature rats were used for this purpose and the method employed was similar to the one used by Cori (2) in his study of the absorption of sugars. Applied to the study of solid foods, instead of solutions, this method has certain limitations, but the results were nevertheless of some interest.

The method was briefly as follows: To each of five or six rats which had fasted 48 to 72 hours there was fed an exactly weighted amount of cereal cooked uniformly for fifteen minutes. The amount consumed was usually about five grams, equivalent to about a gram of the dry cereal. The exact time when the meal was consumed was noted in each case, and at varying intervals thereafter one of the group was sacrificed and the cereal remaining in the stomach and in the small intestine was recovered, ligatures being placed about the cardiac, pyloric, and ileocolic sphincters before the alimentary tract was removed. As a measure of the amount of cereal present a determination of starch seemed most trustworthy, and for a basis of calculation the same determination was made upon a portion of the original cooked cereal.

The determination of starch was made as follows: To the cooked cereal, or gastro-intestinal contents, about 250 cc. of water and 5 cc. of concentrated HCl were added and this mixture was boiled under a reflux condenser for 3 hours. After cooling, neutralizing, and making up to a definite volume, an aliquot was analyzed for reducing sugar by the method

of Bertrand with one modification: the precipitated cuprous oxide was separated and washed by centrifugation instead of by filtration. In the hands of two workers the procedure went smoothly and rapidly and the possibility of reoxidation was reduced to a minimum. When the duplicate analyses failed to agree, additional determinations were made.

Since the animals of a series were not of identical weight, (usually 150 to 200 grams) the figures for cereal (starch) fed and recovered were all put on the basis of 100 gram weight of rat for the purpose of comparison. The amounts of cereal recovered from the stomach and small intestine were each divided by the amount fed and the quotients, expressed in terms of percentage, thus gave a measure of the amount of cereal still present in the stomach and remaining unabsorbed in the intestine. More than 100 animals were used in the entire series.

Before the results are discussed in detail two limitations of the method must be mentioned. The first concerns variations in the motility of the alimentary tract which are more pronounced with respect to solids than with respect to liquids. Furthermore, different animals required variable lengths of time to consume the meal. Some took 15 minutes; others required thirty. Insofar as it was possible to do so, these differences were equalized by using the slowly eating animals for the three and four hour examinations; animals requiring a longer time were omitted from the series. These and other biological variations could not be ruled out except by sufficient observations to treat statistically.

A second limitation in the precision of the method concerns the validity of the figures for reducing sugar as an index of the amount of starch and cereal involved. This applies less to the original cereal sample than to the partially digested gastro-intestinal contents. Glucose is presumably absorbed as rapidly as it is produced by digestion, but the method makes no distinction between starch and its partial decomposition products, the lower dextrans and maltose. The amount of material available was too small to permit a separation of these forms of reducing sugar. Despite these sources of error, starch was a better basis for this work than any of the other cereal constituents. Even in a 48-hour fasted rat the coecum contains considerable amounts of potential reducing material; hence the coecum was omitted from consideration.

The cereals studied were Wheat Endosperm (3), "Whole Wheat" and Pre-cooked Oats. The rates of their passage from the stomach and of the absorption of their starch content are shown in the accompanying charts, in which the amounts of cereal present in stomach and intestine are plotted against time. In the construction of these smoothed curves all the values were used except a few isolated ones shown at the extreme right

in some of the charts. Through delayed motility a residuum of starch was still present in these animals and values could not be put into the averages.

The curves in Charts 1, 2, and 3 represent the gradually decreasing amounts of the cereal foods recovered from stomach and small intestine and from both together, *i.e.*, the rate of passage from the stomach and the rate of absorption in the intestine.

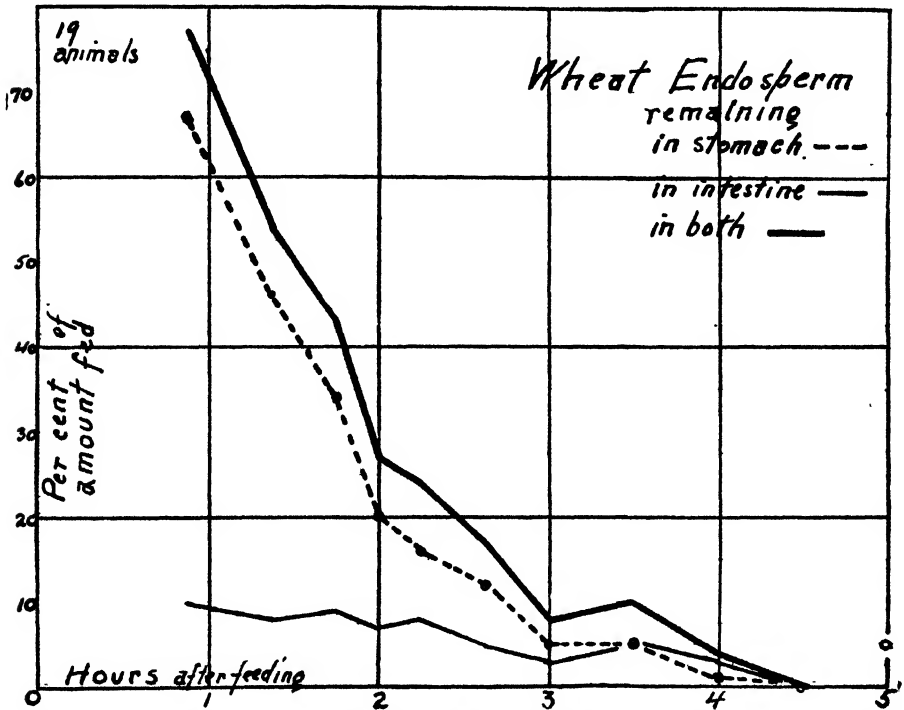


CHART 1.—Average percentage amounts of wheat endosperm remaining in the stomach and in the intestine of rats at different intervals after feeding.

A comparison chart is not necessary to demonstrate that the rate of absorption of Wheat Endosperm was slower than that of "Whole Wheat" during the first two hours. Thereafter they were practically parallel. The relations in the first two hours are, on first consideration, the reverse of what might be expected on the basis of the usual conception that the larger the food particles, the slower they are to leave the stomach. However even a naked-eye examination of the two cereals showed that in Wheat Endosperm the grains were of fairly uniform size while the bran-containing cereal had some large and some very small particles. Further, and as a matter of cooking experience, the presence of bran flakes prevents cereal from coagulating into a solid gummy mass when cooked, as is the case with a bland cereal. Conditions would thus be favorable in the other

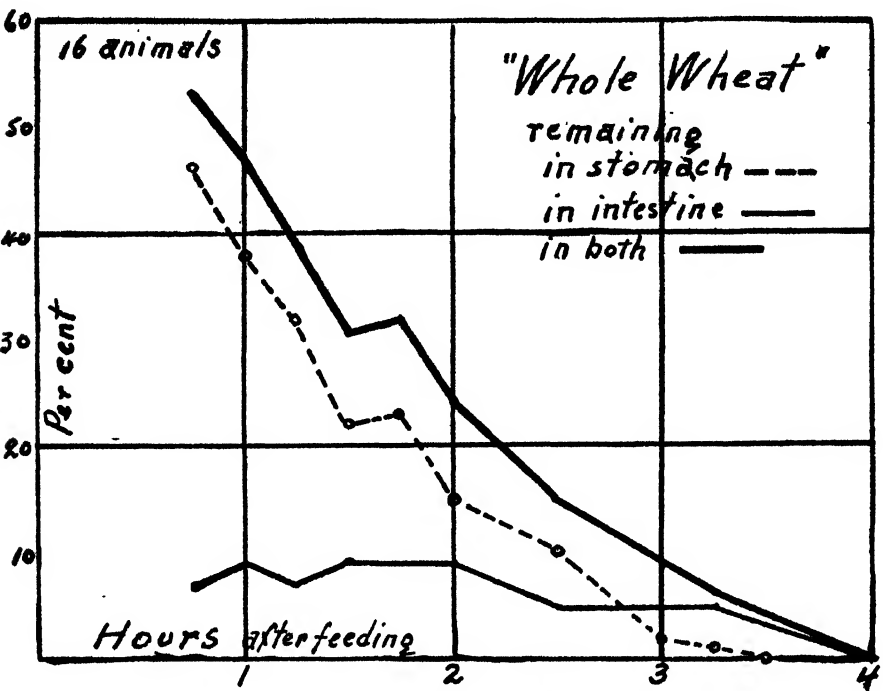


CHART 2.—Average percentage amounts of "whole wheat" remaining in the stomach and intestine of rats at different intervals after feeding.

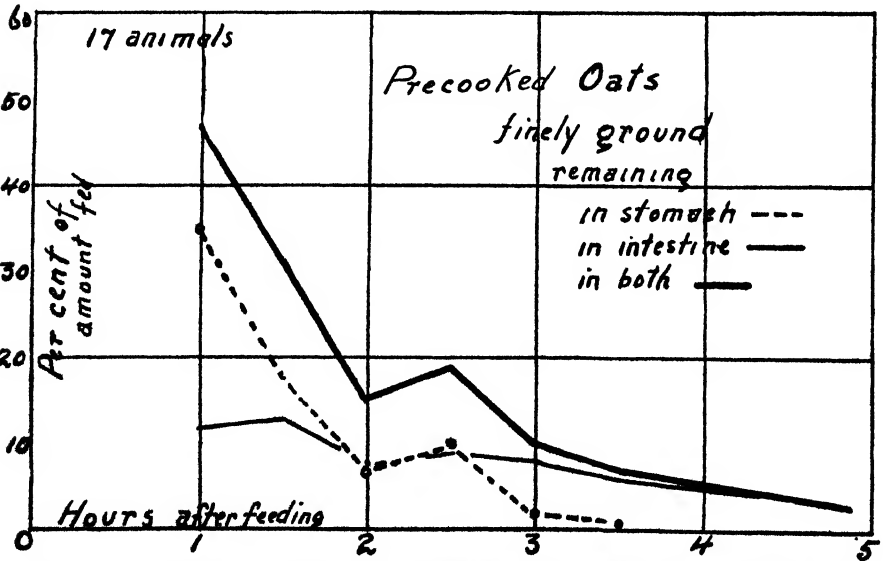


CHART 3.—Average percentage amounts of precooked oats remaining in the stomach and intestine of rats at different intervals after feeding.

for the early departure of the smaller starch particles upon the measure of which the whole experiment depended.

The difficulties encountered in the examination of Precooked Oats tended to confirm this interpretation. In order to assure uniform samples for the parallel analyses it was felt necessary to grind this cereal to a fine flour, and as the chart reveals, the stomach emptying time of this cereal, despite its higher protein and crude fiber content, was shorter than that of either of the others. The longer time required for its complete

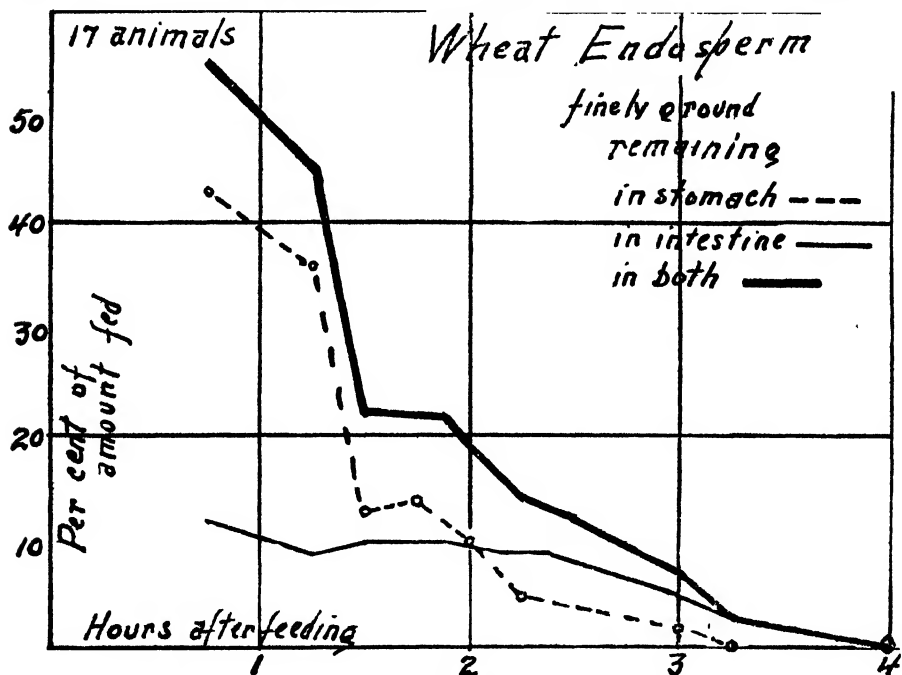


CHART 4.—Average percentage amounts of wheat endosperm (finely ground) remaining in the stomach and in the intestine of rats at different intervals after feeding.

disappearance from the small intestine is in keeping with the usual opinion expressed in the phrase that it "stays by one longer."

It became necessary, therefore, to try the two wheat preparations similarly ground to a fine flour. The data from these tests are given in Charts 4 and 5. That they leave the stomach more rapidly in this form than when used directly from the package is shown in Charts 6 and 7. Finally on Chart 8 are the curves for the emptying time of the stomach for all three breakfast cereals in finely ground form. Within the limits of the method, the three curves are in good agreement and we have, apparently, further proof of the generally accepted fact just mentioned that the more finely divided a food the more rapidly it leaves the stomach.

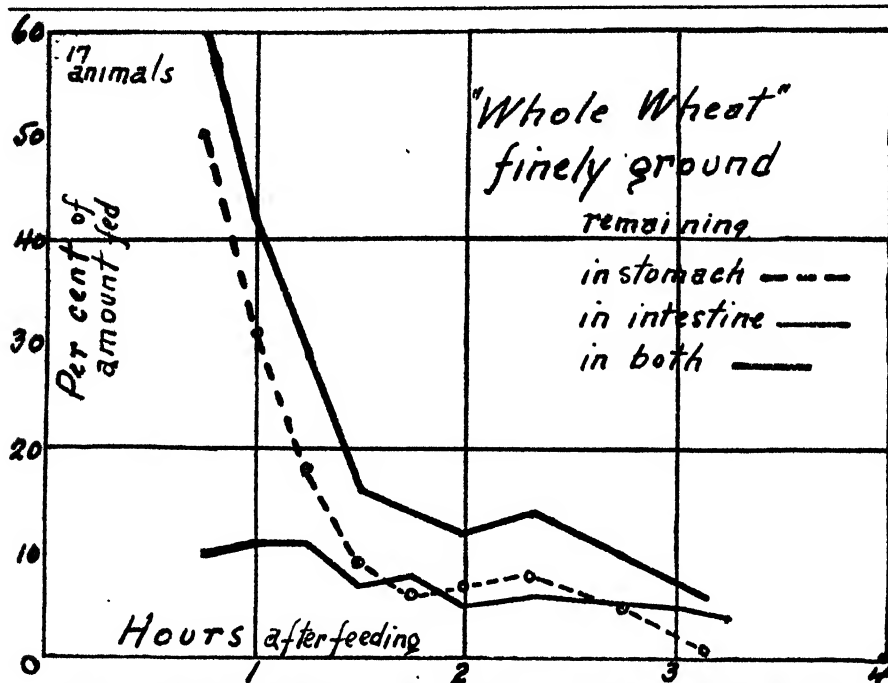


CHART 5.—Average percentage amounts of "whole wheat" (finely ground) remaining in the stomach and in the intestine of rats at different intervals after feeding.

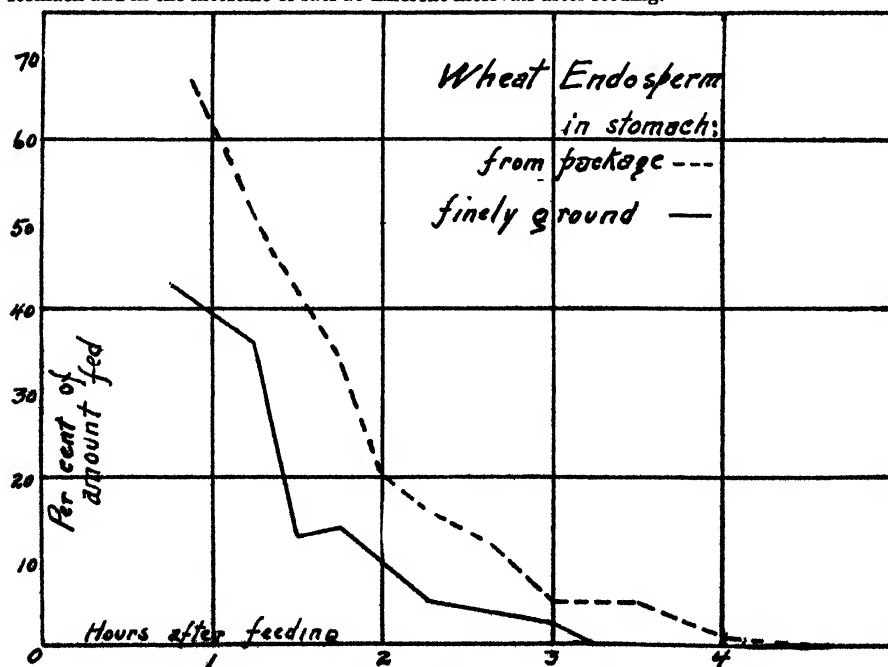


CHART 6.—Average percentage amounts of wheat endosperm remaining in the stomach at different intervals after feeding, when fed as obtained from the package and when fed after being finely ground.

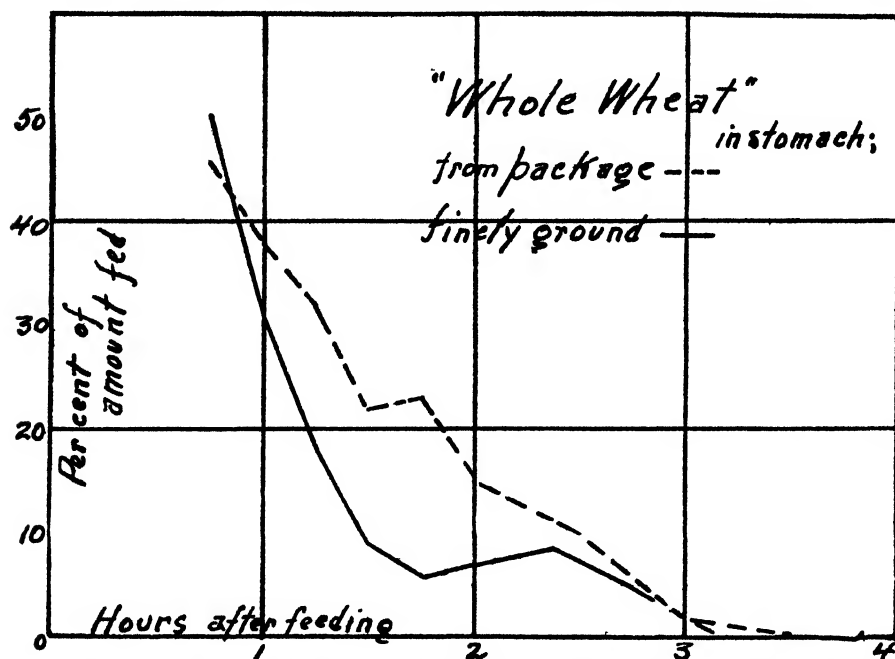


CHART 7.—Average percentage amounts of "whole wheat" remaining in the stomach at different intervals after feeding, when fed as obtained from the package and when fed after being finely ground.

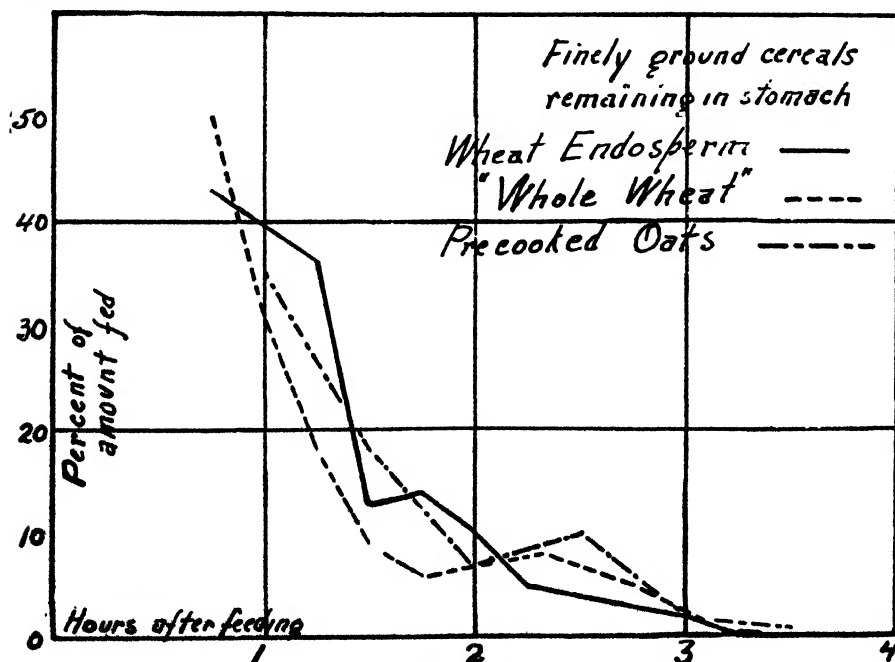


CHART 8.—Average percentage amounts of three different cereals remaining in the stomach at different intervals after feeding. All finely ground before feeding.



Finely divided food spares the stomach and places more of the burden of digestion upon the intestine.

In view of the apparent influence of the size of particles on the stomach emptying time, the effect of adding bran to the wheat endosperm cereal was determined. This was done on 12 animals with the finely ground material. The number of animals used was hardly adequate to allow of definitive conclusions but the addition of 15 to 20 per cent of bran produced surprisingly little change in the stomach emptying time. During the first two hours the curve for the finely ground material plus bran was less than 5 per cent above the zone for the cereals shown in Chart 8 and was well within it thereafter. The same statement may be made regarding the amount of cereal found in the small intestine during the same periods.

If these general results obtained on rats are valid when applied to the human organism, it is apparent that the differences in rates of passage and absorption among the forms of cereals studied are of little importance for a well person. The slightly longer digestion time required by oats is of small consequence in a normal individual. So also is the retarding effect of the presence of bran; indeed, the presence of a small amount of roughage seemed to facilitate the emptying of the stomach (cf. Charts 1 and 2) perhaps by aiding the breaking up of a compact gelatinous mass. The increased rate of stomach emptying produced by fine grinding of the product (Charts 6 and 7) may be credited to the same influence and could perhaps be secured by longer cooking.

#### SUMMARY

By a method described, the rate of passage of cereals from the stomach and the speed of absorption from the intestinal tract of rats were studied. Two wheat cereals, one bland, (Wheat Endosperm) the other containing a small amount of bran ("Whole Wheat"), and Precooked Oats were employed, after 15 minutes cooking. When finely ground, the cereals left the stomach more rapidly than in the granular manufactured form probably because when finely comminuted there was less tendency to form a gummy gelatinous mass. The addition of bran had little if any effect in slowing the rate of stomach emptying in the case of the finely ground materials; there was evidence that the bran particles might even accelerate stomach passage of a compact and glue-like cereal preparation. Physical consistency, rather than the presence of roughage, seems to determine the time of sojourn of cereal in the rat's stomach.

#### BIBLIOGRAPHY

1. Murlin, J. R., and O. H. Gaebler, to be published as paper IV of the series.
2. Cori, C. F., *Jour. Biol. Chem.* 1925, LXVI, 691.
3. Murlin, J. R., W. R. Line, H. A. Piper, and H. B. Pierce, *This Journal*, 1929, II, 83.



# THE PAIRED-FEEDING METHOD IN NUTRITION EXPERIMENTS AND ITS APPLICATION TO THE PROBLEM OF CYSTINE DEFICIENCIES IN FOOD PROTEINS

BY H. H. MITCHELL AND JESSIE R. BEADLES

*(From the Dept. of Animal Husbandry, College of Agriculture,  
University of Illinois, Urbana, Illinois)*

Received for Publication—July 8, 1929

METHODS of research may be divided roughly into methods of measurement and methods for the control of those conditions under which measurements are to be taken. In animal experimentation, the methods of measurement employed include methods of collecting gaseous, liquid, and solid excreta, calorimetric methods, methods of chemical analysis, methods of determining physical properties, such as temperature, and methods of taking weight and dimensional measurements. The importance of accuracy in this type of method is self-evident. For example, in biochemical investigations, the value of experimental results is recognized as dependent primarily on the accuracy of the chemical methods used. Experiments employing analytical methods of little, or even of questionable, accuracy may be practically obliterated from the literature by the mere demonstration of such a state of affairs.

In the biological sciences in particular, methods for the control of those conditions under which measurements are to be taken are fully as important as the methods of measurement themselves, and in many phases of nutrition these methods assume a paramount position. The rapid expansion of the science of nutrition in the last twenty years is largely a direct consequence of the introduction of a method of controlling the nutrient intake of experimental animals by means of synthetic diets composed largely of individually purified constituents. The use of such diets has permitted the effective study of each known dietary factor with reference to any animal function. It has also led to the demonstration of the existence of unknown dietary factors and to their characterization by systematic investigation. The biological analysis of foods is an outgrowth of this factorial method of study.

But unfortunately the effectiveness of methods of control is not so readily ascertained as the effectiveness of methods of measurement, and consequently their imperfections are not so apparent nor so clearly demon-

strable. Frequently the evaluation of a method of experimental control is more a matter of logic than of laboratory investigation. It is perhaps for this reason that faulty methods of control persist in common usage more often and for a longer time than faulty chemical methods. The employment of improperly controlled experimental procedures means not only ineffectual experimentation, yielding results defying interpretation, but also the acceptance as facts of experimental interpretations that are not securely established. Even though an experimental interpretation of this character ultimately proves to be correct, it must be reassessed before it can possess any claim to finality.

It is a relatively easy matter to control an experiment with reference to the peculiarities of individual behavior by employing a number of animals properly selected, or by letting each animal be its own control in a succession of properly selected periods of experimental treatment and observation. The equalization of environmental conditions of temperature, humidity, light, shelter, confinement, etc., among the different animals or groups of animals to be compared is generally recognized as an important requisite to a clear solution of the problem under investigation. In nutrition experiments the composition of the experimental diets is often the main item of concern, and frequently the greatest care is taken in the compounding of rations to the end that between any two rations only one constituent shall be variable. But the control of the amount of food consumed has always been the most neglected and the most baffling problem in nutrition investigations, and the failure to control the food intake of experimental animals has been the direct cause of much ineffective experimental work.

In 1912, F. G. Hopkins published a report of feeding experiments on rats illustrating the importance of the food consumption of animals in the interpretation of such data (1). This paper possesses the historical importance of foreshadowing the discovery of the vitamins and constitutes Hopkins' claim to recognition in this connection. In the experiments reported, young rats were offered synthetic diets made up of isolated proteins, fats, carbohydrates, and a salt mixture. Other rats were offered the same ration, but with the addition of a small amount of milk. The rats receiving no milk soon failed to grow, while the rats receiving the milk addendum, amounting to 4 per cent or less of the dry matter in the ration, grew normally and continuously. The food intakes of the rats were not controlled, but their importance in the interpretation of the experiments was fully recognized. Almost 10 pages of this report, or almost exactly one third of the text, are concerned with the question of the relation of the growth secured to the amount of food consumed. Only after he was

able to assure himself, necessarily by indirect means, that the difference in the growth observed between the milk rats and the non-milk rats could not have been due solely to the difference in the amounts of food consumed, was Hopkins prepared to conclude that the milk addendum exerted a direct effect upon the growth processes.

This classical experiment testifies to the fact that the proper control of nutrition experiments should extend beyond the control of the composition of the diet to the control of its consumption by experimental animals. The painstaking care of Hopkins in making the most of a confusing situation, apparently not anticipated in the planning of the experiment, established his conclusion with what appears to be a high degree of probability, but undoubtedly the interpretation would have been more direct and more convincing if the food intake of his milk rats had been restricted to that of his non-milk rats. If one ration is superior to another in the support of an animal function such as growth, its superiority should be evident when the intake of both rations by comparable animals is the same, either absolutely or in proportion to some determinant of food requirements, such as body weight, or a mathematical function of body weight. In fact, such an equality of food intake is essential to a clear-cut demonstration of a difference in nutritive value.

#### THE PAIRED-FEEDING METHOD

The problem of the control of food intake is thus an important one in animal experimentation; it is also a difficult one to solve. We propose to consider a plan of food control that seems hopeful of success, and that actually, in a number of laboratories, has given results capable of only one interpretation, surely an unmistakable earmark of effective experimentation.

The first experiment in the literature, in so far as we have been able to discover, in which this method of food control was used, is a cooperative experiment planned by the late Dr. Armsby in 1917 and sponsored by the National Research Council. The purpose of these investigations, which involved eight state agricultural experiment stations, was to compare the effect upon the growth of calves, as measured by body weight, body dimensions, and the rate of nitrogen retention, of two rations containing different percentages of essentially the same protein mixture. The point of immediate interest is the method by which this comparison was to be effected. To quote from the plan of the experiment:

"The plan contemplates the comparison of the animals by pairs, one animal of each pair receiving the high-protein and one the low-protein ration. The two animals of each pair should be

of substantially the same breed (not necessarily full-bloods) and as nearly as possible of the same age and weight at the beginning of the experiment.

"It is desired that as many pairs be used as is consistent with the keeping of individual records. Lot records are of far less value. The more nearly alike the several pairs are the better, but uniformity in this respect is not so essential as between the two animals of each pair." (2).

Omitting the details of the management of the calves during the growing period, it is necessary to point out only two items of control as bearing upon the question under discussion. The first item is that "if the proportion of roughage in the rations proves to be too great, the quantity of oat straw may be reduced *for both animals of a pair* by the same amount per 1000 pounds live weight." The second item refers to the adjustment of the energy supply of the animals "in order, on the one hand, to prevent fattening or, on the other, to maintain good condition. This may be effected by varying the amount of starch used, but *the energy supply of both animals of a pair must be kept the same per 1000 pounds live weight.*" The italics are Armsby's.

Although the results of these experiments were not sufficiently significant nor concordant to afford any definite conclusion concerning the relative growth-promoting value of the high-protein and low-protein rations, the fault did not lie in the plan of the investigations, but in the indifferent success with which the plan was carried out. Either intentionally or inadvertently the net energy intakes of the paired calves were not equalized as directed, so that the observed differences in growth cannot be interpreted with reference to the difference in protein intake only.

The determination of the nutritive deficiencies of rations of which experimental animals, either immediately or eventually, will not consume enough for the maintenance of weight, has proved to be a difficult problem. The results obtained cannot be interpreted on the basis of their face value. In laboratory investigations there is a marked tendency to assume that the refusal of rations by experimental animals is proof *ipso facto* that they are inadequate in one or more nutritive constituents. But this is not a critical interpretation. In experiments of this character, the paired-feeding method seems to offer marked advantages, and in actual experimental work had proved its value.

Essentially this plan of food control has been employed with highly significant results in studies of the physiological effects of rations deficient in those water-soluble vitamins until recently unified under the term "vitamin B." The particular need of control of food consumption in such investigations resides in the fact that the appetite of animals subsisting upon these deficient rations ultimately is seriously impaired, even to the extent of total refusal of food. The question arises, therefore, to what

extent are the physiological effects produced due to a specific vitamin hunger, and to what extent are they due to a generalized undernutrition or even to inanition. Strange as it may seem, this point has not been considered in the large majority of all investigational work of this character on vitamins.

One of the physiological effects ascribed to a lack of the so called "vitamin B" is a severely depressed basal heat production. In 1922, and again in 1924, Gulick (3) investigated this particular relation by the paired-feeding method. He found that the basal metabolism of rats was indeed severely depressed to a level less than 70 per cent of the normal by subsistence upon a "vitamin-B-free" diet. But their food intakes were also severely depressed. If for each test rat, a litter mate control was fed upon the same diet plus a small amount of "vitamin-B" supplement (dried yeast), and in the same amounts, restricted in accordance with the diminishing food consumption of the test rat, a severe depression in basal metabolism also resulted, similar in magnitude to that observed in the rat receiving no "vitamin B." Thus, the depression in metabolism in rats receiving a ration deficient in "vitamin B" was shown conclusively to be unrelated to the vitamin deficiency, but to be the result of a generalized undernutrition.

The results of Gulick were confirmed four years later by Drummond and Marrian (4), who showed that the nutritive failure in rats following a deficiency of "vitamin B" is virtually identical with that resulting from starvation, and that the starved rats exhibited nerve lesions indistinguishable from those found in "vitamin-B" deficient rats.

In the following year Kon and Drummond (5) applied the paired-feeding method to a similar study on pigeons. The distinction between the effects of inanition and those of vitamin deficiency was made possible by the control of the food intake of both of a pair of birds of equal weight to the amount voluntarily eaten by the one on the deficient diet. The experiment, which involved a number of such pairs, proved that most of the symptoms attributed to a dietetic deficiency of "vitamin B," except adrenal hypertrophy and acute nervous symptoms, were present equally in the control bird and its pair mate, even the degenerative changes in the peripheral nerves that have been considered a specific result of a lack of the antineuritic vitamin. In commenting upon this remarkable outcome of their experiment, Kon and Drummond say, "No existing theory is considered as giving a satisfactory explanation of the role of vitamin B in the normal organism, or of the true nature of the conditions induced by its deficiency."

The paired-feeding method has recently been used by Rose, Stucky, and Cowgill (6) in studies of the relationship between the depressed gastric motility observed in dogs subsisting upon "vitamin-B-free" food and the deficiency in vitamins. Again the effects of undernutrition (as well as of hunger) in the etiology of this condition were established.

When rations are offered to experimental animals *ad libitum*, many factors, known and unknown, contribute to the determination of the amounts of feed actually consumed. Among the known factors may be mentioned the flavor, texture, nutritive completeness and available energy content of the ration. If two rations are being compared with reference to their inherent nutritive values, a difference in the intake of each by the experimental animals is to be expected. But by permitting a difference in food intake to develop between animals whose growth is to be compared, the interpretation of the difference in growth in terms of the make-up of the rations is difficult and may even become impossible. The argument is well illustrated by experiments concerned with the value of common salt as a supplement to cereal rations. The condimental value of salt is well known, so that the mere demonstration that animals grow better when given cereal rations plus salt than when given cereal rations alone may mean simply that a more liberal consumption of the rations has thus been induced. However, at the Nutrition Laboratory of the University of Illinois (7) a ration based mainly upon corn, when supplemented with salt, induced 50 per cent faster gains in weight of both rats and chickens than the salt-less ration, even when the food consumption of the paired animals was kept the same. The deficiency of corn in either sodium, or chlorine, or both, was thus clearly established.

A similar paired-feeding investigation with growing pigs, concerned with the supplemental value of ferrous sulfate (copperas) added to rations both high and low in iron, was reported in 1927 by Carroll and Mitchell (8).

If the food intakes of the pair mates have been strictly equalized throughout the period of experimental feeding, the paired-feeding method is capable of a high degree of accuracy, such as is needed in the detection of small differences in the nutritive balance of rations. For 4, 5, or 6 pairs the outcome of the test should be identical in all pairs, if a significant difference in nutritive value exists between the two rations tested. The results of the method, however, are peculiarly susceptible to simple statistical treatment, since there can be no suspicion that the selection of the animals interferes in the slightest with the randomness of the differences between pair mates in growth, blood composition, bone composition, or other measurement taken. In fact, the more successful the selection of pair mates has been in the direction of equality of initial capacity to grow

and develop on the basal ration, the more clear-cut will be the statistical interpretation. In contrast with this is the ordinary group-feeding method. Here the more effective the selection of animals in equalizing the growth capacities of the groups to be compared, the more the randomness of the gains obtained within groups is disturbed, and the less applicable become the ordinary statistical methods in the interpretation of the results secured. And yet, without the aid of statistical methods the results of the ordinary group-feeding experiment frequently cannot be precisely interpreted.

#### ITS APPLICATION TO THE PROBLEM OF CYSTINE DEFICIENCIES

The paired-feeding method is admirably adapted to the problem of the determination of the amino acid deficiencies of proteins and mixtures of proteins, upon which their supplementary relations depend. Some work has been done upon the cystine deficiencies of a number of food products and it is this work upon which a detailed report will be made here.

A preliminary survey of a number of foods was first made, using only 3 or 4 pairs of rats in each case. These foods were skim milk powder, white bread, potato, navy beans, beef muscle, and canned green garden peas. The bread was a bakery product containing milk, and was dried only. The potatoes were baked in an oven, peeled, sliced, and dried at a low temperature in a current of warm air. The navy beans were soaked in distilled water, boiled thoroughly, and dried without waste. The beef muscle was obtained as round steak. The lean was freed of all visible fat, ground, dried, and extracted with ether. The peas were a local brand of canned garden peas. They were boiled for about 15 minutes and dried without waste.

After drying and grinding, all foods were analyzed for nitrogen and made up into rations containing approximately 8 per cent of protein ( $N \times 6.25$ ), furnished entirely by an individual food. For each food a second ration was made up containing 0.24 per cent of cystine and approximately 7.76 per cent of protein. The composition of these rations is given in Table I.

Four pairs of rats were started upon each food, one rat in each pair to receive the unsupplemented ration and the other rat the ration containing added cystine. In each pair the rats were of the same sex, frequently of the same litter, and in all cases of the same weight within a few grams. They were kept in individual cages and fed weighed amounts of their respective rations, the food intake being controlled so that the two rats of a pair received as nearly as possible the same amount of food per week, determined by the rat consuming the least. After the first 5 weeks of feeding each rat received an additional daily vitamin supply as cod liver oil and dried yeast or yeast extract. All rats were weighed weekly.



TABLE I  
THE COMPOSITION OF THE RATIONS

	Nitrogen	Protein	All values in percentage by weight											
			A	B	C	D	E	F	G	H	I	J	K	L
Milk powder...	5.735	35.84	22.32	21.65	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Bread.....	2.116	13.23	.....	.....	60.49	58.68	.....	.....	.....	.....	.....	.....	.....	.....
Potato.....	1.683	10.52	.....	.....	.....	.....	76.05	73.77	.....	.....	.....	.....	.....	.....
Beans, navy.....	3.946	24.66	.....	.....	.....	.....	.....	.....	32.44	31.47	.....	.....	.....	.....
Beef, dried.....	14.782	92.39	.....	.....	.....	.....	.....	.....	.....	.....	8.66	8.40	.....	.....
Pea, garden.....	3.286	20.54	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	38.95	37.78
Cystine.....	11.51	.....	.....	0.24	.....	0.24	.....	0.24	.....	0.24	.....	0.24	.....	0.24
Salts, Osborne and Mendel.....	.....	.....	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Butter fat.....	.....	.....	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Sucrose.....	.....	.....	10.00	10.00	10.00	10.00	4.95	6.99	10.00	10.00	10.00	10.00	10.00	10.00
Cellu Flour.....	.....	.....	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Sodium chloride.....	.....	.....	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Starch.....	.....	.....	48.68	49.11	10.51	12.08	.....	.....	38.56	39.29	62.34	62.36	32.05	32.98
Totals.....	.....	.....	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nitrogen.....	.....	.....	1.239	1.264	1.278	1.322	1.273	1.267	1.354	1.263	1.279	1.254	1.333	1.269
Protein.....	.....	.....	7.741	7.901	7.988	8.263	7.956	7.919	8.463	7.894	7.994	7.838	8.442	7.931

One pair of rats on the potato ration was withdrawn from the experiment very soon after it began because of respiratory disease. Also one of the rats on the pea ration died after 4 weeks of feeding.

The results of this preliminary experiment have been condensed and summarized in Table II. The items listed in this table are self-explanatory except the last, described as "comparison of weekly gains." The corresponding figures give for each rat the number of weeks for which its gain surpassed that of its pair mate. In the case of equal gains, 0.5 is credited to each pair mate.

In the case of beef muscle and white bread, the cystine supplement was evidently ineffective in promoting more rapid gains. In the former case in 3 of the 4 pairs the cystine rat gained the more, but of the 48 weekly comparisons for all pairs the control rat was ahead in 22.5 cases and the cystine rat in 25.5 cases, a close approximation to equality. In the white bread experiment, the control rat gained the more in each pair, but this may well have been a chance result with such a small number of pairs. If an event may occur with equal probability in either of 2 ways, then in a random selection of 4 trials, the frequencies of its occurrence in one way 0, 1, 2, 3, and 4 times is given by the expansion of the binomial distribution  $(\frac{1}{2} + \frac{1}{2})^4$ .

Its occurrence 4 times in either way would result from chance once in 16 trials. Hence, with 4 pairs of rats, a consistent outcome favoring either the control or the test rats would result from chance only, twice in 16 trials. A detrimental effect of cystine in this case seems improbable in view of the results on other pairs, so that this particular outcome may well be considered fortuitous. Of the 59 weekly comparisons among these 4 pairs of rats, 34 favored the control rat and 25 the cystine rat.

Among the three pairs of rats on the potato ration, the total gains of the cystine rats in all cases exceeded those of their pair mates, while of the 48 comparisons of weekly gains, the cystine rat was favored in 33 and the control rat in only 15. The former result cannot be handled statistically in a satisfactory manner because only 3 pairs were used, but the latter result may be analyzed further on the assumption that, if the cystine supplement is without value, the chances are even that in any one week on equal intakes of food the gain in weight from either ration will exceed that from the other. Hence, in the present case, if the nitrogen compounds of potato were not improved in growth-promoting value by the cystine supplement, the ideal outcome of 48 comparisons of the weekly gains of paired rats would be 24 favoring the control rat and 24 favoring the cystine rat. The question at issue is whether the deviation from this

TABLE II  
THE CYSTINE DEFICIENCIES OF FOOD PROTEINS AS DETERMINED BY THE PAIRED-FEEDING METHOD  
(All weights in grams)

Food investigated	Data	Pair 1		Pair 2		Pair 3		Pair 4	
		Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Peas, cooked.....	Initial weight	71	65	130	132	41	40	38	36
	Final weight	79	92	130	178	51	59	67	94
	Gain	8	27	0	46	10	19	29	58
	Total food	288	281	485	488	109	109	273	281
	Days on food	77	77	77	77	28	28	77	77
Beef muscle, fat-free.....	Comparison of wk. gains	1.5	9.5	0.5	10.5	0	4	1	10
	Initial weight	51	53	45	43	56	56	68	64
	Final weight	146	169	159	158	163	160	197	217
	Gain	95	116	114	115	107	104	129	153
	Total food	542	533	508	507	562	555	648	646
Navy beans, cooked.....	Days on food	84	84	84	84	84	84	84	84
	Comparison of wk. gains	4.5	7.5	5.5	6.5	7.5	4.5	5	7
	Initial weight	62	60	57	60	63	62	63	61
	Final weight	80	85	80	94	74	83	80	91
	Gain	18	25	23	34	11	21	17	30
	Total food	268	274	302	299	250	261	301	301
	Days on food	77	77	77	77	77	77	77	77
	Comparison of wk. gains	4.5	6.5	4	7	5	6	3	8

TABLE II (Continued)  
THE CYSTINE DEFICIENCIES OF FOOD PROTEINS AS DETERMINED BY THE PAIRED-FEEDING METHOD  
(All weights in grams)

Food investigated	Data	Pair 1		Pair 2		Pair 3		Pair 4	
		Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Potato, baked.....	Initial weight	52	52	46	46	62	62		
	Final weight	76	88	119	139	115	148		
	Gain	24	36	73	93	53	86		
	Total food	504	498	666	666	701	700		
	Days on food	112	112	112	112	112	112		
White bread.....	Comparison of wk. gains	6	10	6	10	3	13		
	Initial weight	51	50	55	56	64	64	55	55
	Final weight	98	95	79	73	104	103	96	84
	Gain	47	45	24	17	40	39	41	29
	Total food	477	476	304	301	472	472	426	423
Skim milk.....	Days on food	112	112	77	77	112	112	112	112
	Comparison of wk. gains	9.5	6.5	6	5	9	7	9.5	6.5
	Initial weight	52	51	45	46	50	50	49	48
	Final weight	187	205	180	214	194	234	167	163
	Gain	135	154	135	168	144	184	118	115
	Total food	815	810	797	793	1000	1006	746	753
	Days on food	99	99	99	99	112	112	99	99
	Comparison of wk. gains	5.5	8.5	3	11	3.5	12.5	8.5	5.5

ideal, obtained in this particular experiment, *i.e.*,  $33 - 24 = 9$ , could reasonably result from the operation of chance, which would have determined the deviation if the cystine supplement were without nutritive effect. This again is a proposition involving the assessment of probabilities by means of the binomial distribution. The standard deviation of the frequency distribution of the outcome of 48 events, each of which may result with equal probability in either of two ways, is given by the expression  $\sqrt{0.5 \times 0.5 \times 48}$ ,<sup>1</sup> which is equal to 3.46. The deviation of 9 from the expected outcome is 2.6 times this standard deviation. It would occur by chance only once in 107 trials,<sup>2</sup> so that this method of analysis indicates clearly that cystine actually supplemented the potato ration and induced more rapid gains than would have resulted otherwise on the same intake of food.

Of the four pairs of rats on the navy bean ration, the cystine rat gained distinctly more rapidly in all pairs, though the rate of gain was extremely small in all cases. This result would have been obtained by chance once in 16 trials and, therefore, in itself it proves nothing. Of the 44 comparisons of weekly gains in all 4 pairs, 27.5 favored the cystine rat, a deviation of 5.5 from the expectation, if chance alone had determined the outcome. The standard deviation in this case, computed as explained above, is 3.32; the deviation of 5.5 is only 1.66 times this value, an outcome that may have resulted from chance once in 10 trials. This method also fails to establish a sound basis for the belief that cystine has effectively supplemented the proteins of navy beans. However, neither of these methods of analysis takes full advantage of the data, since they consider only the sign of the differences in the gains of pair mates, not their magnitudes. In view of the inconclusive nature of the results obtained thus far, the analysis should be continued further on the latter basis. The average of the 44 weekly differences in the gains of paired rats is 0.932 gram in favor of the cystine animal. The standard deviation of these differences is 2.94 grams, and the probable error of the average difference, 0.30 gram. Since the average difference is more than 3 times its probable error, it may be considered significant. It appears probable that cystine has effectively supplemented the proteins of navy beans, but that, under the conditions of this experiment, the effect was too small to be conclusively demonstrated with only 4 pairs of rats.

The results with skim milk were rendered somewhat inconclusive by the fact that for one pair of rats the control animal gained 3 grams more

<sup>1</sup> See R. A. Fisher: *Statistical Methods for Research Workers*. Edinburgh and London, 1928. Chapter III.

<sup>2</sup> According to Davenport's Table of Values of the Normal Probability Integral.

than the cystine animal in 99 days of feeding. Of the 58 comparisons of weekly gains, however, the cystine rat was favored in 37.5, a deviation of 8.5 from the mid-value of 29. The standard deviation in this case ( $\sqrt{0.5 \times 0.5 \times 58}$ ) equals 3.81. The deviation is 2.23 times its standard deviation, and would be obtained by chance only once in 39 trials. Proceeding further with these differences, the average difference in weekly gain between paired rats with its probable error is  $+1.40 \pm .43$ , the sign indicating that the cystine rats showed the larger average gain. In spite of the erratic behavior of one pair of rats, the analysis may be taken to mean that, with a high degree of probability, cystine effectively supplemented milk proteins in their growth-promoting value.

The 4 pairs of rats on the ration containing, as its source of protein, canned garden peas, gave unmistakable evidence that the dietary protein was deficient in cystine. In spite of the very slow gains made, the effect of the added cystine was clear. In all 4 pairs of rats, the total gain of the cystine rat exceeded considerably the total gain of its control. Of the 37 weekly comparisons of gains between pair mates, 34 favored the cystine rat and only 3 the control, an outcome extremely improbable by the operation of chance factors only.

It seemed desirable to repeat the tests upon some of these foods with a larger number of pairs of animals, both as a further test of the method, and as a more satisfactory test of the foods. The fact that one pair of rats on the skim milk diets gave evidence contrary to that of the other 3 pairs, the latter evidence confirming the conclusion of Sherman and Merrill (9) that milk proteins are deficient in cystine, made it seem desirable to repeat the test on this food. Although the data on garden peas were remarkably consistent and clean cut, the conclusion that this variety of *Pisum sativum* possesses proteins deficient in cystine, while, according to Finks, Jones, and Johns (10), the field pea belonging to the same species either in the uncooked or cooked condition, does not, would seem to need further investigation.

Accordingly the experiment with skim milk was repeated with 9 pairs of rats, and that with garden peas with 6 pairs of rats. Another brand of canned garden peas was used in the latter test. The results of the tests are summarized in Tables III and IV.

In the milk test, the rat receiving the cystine supplement gained more than its pair mate in 10 weeks of feeding in each of the 9 pairs. This result would have been obtained by chance only once in 512 trials. Of the 90 comparisons of the weekly gains of paired rats, 73.5 favored the rat receiving the cystine supplement. The ideal result, if chance alone operated,

TABLE III  
THE VALUE OF CYSTINE AS A SUPPLEMENT TO THE PROTEINS OF WHOLE MILK  
(All weights in grams)

	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6		Pair 7		Pair 8		Pair 9	
	Con- trol	Cys- tine	Con- trol	Cys- tine	Con- trol	Cys- tine	Con- trol	Cys- tine	Con- trol	Cys- tine	Con- trol	Cys- tine	Con- trol	Cys- tine	Con- trol	Cys- tine	Con- trol	Cys- tine
Initial weight	57	56	55	52	51	51	47	45	40	40	40	41	41	37	32	34	34	34
Final weight	163	177	153	152	146	164	141	165	152	189	148	173	135	171	128	164	137	151
Gain	106	121	98	100	95	113	94	120	112	149	108	132	94	134	96	130	103	117
Total food	717	712	599	606	662	657	613	611	619	622	594	594	595	595	575	578	544	541
Days fed	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
Comparison of wk. gains	4	6	3.5	6.5	3	7	1.5	8.5	0	10	1.5	8.5	1	9	0	10	2	8

TABLE IV  
THE VALUE OF CYSTINE AS A SUPPLEMENT TO THE PROTEINS OF COOKED GARDEN PEAS  
(All weights in grams)

	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6	
	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine	Control	Cystine
Initial weight	117	115	103	100	107	108	52	50	50	47	46	45
Final weight	150	168	117	132	141	166	90	101	87	98	83	97
Gain	33	53	14	32	34	58	38	51	37	51	37	52
Total food	755	745	550	565	711	721	398	398	378	369	395	387
Days fed	113	113	113	113	113	113	106	106	106	106	106	106
Comparison of wk. gains	5.5	10.5	5	11	3	13	4	11	5	10	3	12

would be 45, and the significance of the deviation of actual and theoretical results, *i.e.*,  $73.5 - 45 = 28.5$ , must be assessed. The standard deviation in this case is  $\sqrt{0.5 \times 0.5 \times 90} = 4.74$ . The deviation is almost exactly 6 times the standard deviation and could thus hardly have resulted from chance. Both methods of analysis, therefore, agree in showing that milk proteins are undoubtedly deficient in cystine, and would be effectively supplemented by cystine-rich proteins.

In the experiment on garden peas, the percentage of pea protein in the rations was initially made approximately 8 per cent, but after 3 weeks of feeding this percentage was increased to 12 per cent because of the slow growth that was being obtained. Neither ration was well eaten and the growth throughout the experiment was very slow. Nevertheless, in spite of these adverse conditions, the paired-feeding method was capable of showing unmistakably that the proteins of cooked green garden peas are deficient in cystine. All six pairs placed the cystine rat ahead of its pair mate in total gain during approximately 15 or 16 weeks of feeding. Such a result would have been obtained by chance only once in 64 trials. Of the 93 comparisons of weekly gains between pair mates, 67.5 favored the cystine rat, a deviation from the chance expectation (46.5) of 21. This is 4.3 times the standard deviation (4.82), and could not be considered within reason to be the resultant of chance factors only.

"Student's" method (11) for analyzing the significance of small groups of differences between test and control observations may also be applied to paired-feeding experiments such as this. The average differences between the total gains of the 6 pair mates is 17.3 grams, and the standard deviation of differences is 3.83 grams. The ratio of the mean difference to the standard deviation, the  $z$  of "Student," is 4.5. With  $z = 4.5$  and  $n = 6$ , according to the table of "Student," the odds are well over 2000 to 1 that the cystine supplement was responsible for the rapid gains of the rats consuming the cystine ration.

In connection with the experiment on garden peas, the digestibility of the pea protein, with and without the cystine supplement, was determined from 7 day collections of feces, once on the 8 per cent protein ration and again on the 12 per cent protein ration. The coefficients of digestibility are summarized in Table V.

Cystine does not seem to have affected the digestibility of the proteins of peas, according to these figures. On the 8 per cent ratio, the average coefficient for the control rats was 79.5 and for the cystine rats 77.8. On the 12 per cent ration, the averages were 80.0 and 80.3, respectively.

It has been demonstrated by Mitchell and Carman (12) that the gains



in weight of growing rats may not be fair measures of the nutritive values of the rations upon which they have been subsisting, because of the fact that gains may vary widely in chemical composition, particularly as regards fat. Does this observation vitiate the significance of the comparisons of gains secured in paired-feeding experiments? In an attempt to

TABLE V  
THE COEFFICIENTS OF DIGESTIBILITY OF NITROGEN OF THE GARDEN PEA  
RATIONS WITH AND WITHOUT THE CYSTINE SUPPLEMENT

	Protein Content of Ration			Protein Content of Ration	
	8 pct.	12 pct.		8 pct.	12 pct.
Pair 1			Pair 4		
Control	83.8	85.1	Control	79.5	83.4
Cystine	74.9	80.3	Cystine	70.2	79.4
Pair 2			Pair 5		
Control	74.2	78.4	Control	77.6	75.2
Cystine	90.2	83.9	Cystine	79.4	76.5
Pair 3			Pair 6		
Control	78.1	82.8	Control	83.7	75.4
Cystine	79.8	83.5	Cystine	72.2	78.3

answer this question, the rats in the milk experiment described above were analyzed after removal of the contents of the alimentary canal. The results of this analysis are summarized in Table VI.

With two exceptions the percentage of protein in the cystine rats exceeded that of their pair mates, and for all pairs the protein content in grams of the cystine rat was greater than that of the controls. It is interesting to note that with Pair 2, in which the control rat and the cystine rat gained approximately the same and attained to almost identical final weights, the carcass of the control rat contained 22.43 per cent of ether-soluble matter, while the cystine rat contained only 14.18 per cent. Also, the weight of protein in the latter animal was appreciably greater than in the former. Evidently on the same kind of food except for the cystine content, and on the same amount of food, the control rat fattened to a much greater extent than the cystine rat. In Pair 7, the reverse seems to have occurred. Among the other pairs of rats the fat content of pair mates did not vary widely. It may be significant that the greatest divergencies among pair mates in the percentage of fat in the carcass, *i.e.*, in

Pairs 2, 3, and 7, occurred in pairs made up from rats of different litters. The rats in Pairs 1, 5, 6, and 8 were litter mates.

It may be concluded that these analytical data substantiate, rather than vitiate, the live weight comparisons of paired rats, and that in the case of Pair 2 they explain satisfactorily an approximate equality of gain in live

TABLE VI

CHEMICAL COMPOSITION OF RATS RAISED ON THE MILK RATION WITH AND WITHOUT CYSTINE

	Empty weight gms.	Percentage Composition				Protein content gms.
		Dry matter	Crude protein	Ether extract	Ash	
Pair 1						
Control	160	41.39	18.81	18.65	4.00	30.1
Cystine	174	41.94	18.19	19.03	3.81	31.7
Pair 2						
Control	151	43.51	16.81	22.43	3.96	25.4
Cystine	150	36.98	18.31	14.18	4.44	27.5
Pair 3						
Control	142	38.68	18.56	15.39	4.28	26.4
Cystine	157	37.93	20.50	13.42	4.19	32.2
Pair 4						
Control	141	41.97	17.25	19.04	4.24	24.3
Cystine	165	42.22	18.19	19.57	3.87	30.0
Pair 5						
Control	152	38.71	18.13	16.40	3.72	27.6
Cystine	180	40.68	18.88	16.37	3.83	34.0
Pair 6						
Control	144	36.75	17.94	13.73	3.84	25.8
Cystine	169	37.55	19.19	13.78	3.99	32.4
Pair 7						
Control	133	39.58	18.25	17.55	4.14	24.3
Cystine	168	45.07	17.25	22.94	3.73	29.0
Pair 8						
Control	126	38.58	18.88	15.09	4.24	23.8
Cystine	162	40.05	19.00	16.41	3.99	30.8
Pair 9						
Control	137	39.28	18.13	16.16	3.81	24.8
Cystine	148	40.33	18.88	16.70	4.23	27.9

weight that is at variance with the belief that cystine has effectively supplemented milk proteins.

### SUMMARY AND CONCLUSIONS

The paired-feeding method has been used effectively in a number of investigations in which the effect of a variable food intake must be eliminated to permit of a clear interpretation of the results with reference to the one deliberately imposed variable. Its results may be readily analyzed by simple statistical methods.

The method is particularly well adapted to a study of the amino acid deficiencies of proteins and of the mixtures of proteins and non-protein nitrogenous compounds occurring in natural foods. In this experiment it has been used in the determination of the existence of cystine deficiencies among a number of food products. The results appear to justify the following conclusions:

The proteins of lean beef and of white bread are not deficient in cystine since their growth-promoting value for rats is not enhanced by supplementing with cystine.

The proteins of navy beans, potatoes, milk, and garden peas are deficient in cystine in supplying the growth requirements of the rat. A repetition of the experiments on milk and garden peas confirmed strongly the results of the preliminary experiments on only 4 pairs of rats.

From the experiments on peas, in particular, it appears that the paired-feeding method is capable of giving clear-cut results under the adverse condition of inadequate food consumption by the experimental animals.

The addition of cystine to the garden pea ration did not modify the completeness of digestion of the nitrogenous compounds contained in it.

Further evidence is presented to show that the fat content of the gains of rats made on the same amounts of rations of nearly identical make-up may differ widely.

### REFERENCES CITED

1. Hopkins, F. G., Feeding experiments illustrating the importance of accessory factors in normal dietaries. *Jour. Physiol.*, 1912, XLIV, 425.
2. Cooperative experiments upon the protein requirements for the growth of calves. *Bull. Nat. Res. Council*, No. 12, 219-288, 1921.
3. Gulick, A., The influence of a beri-beri diet upon the metabolic rate of the white rat. *Proc. Amer. Physiol. Soc.*, *Amer. Jour. Physiol.*, 1922, LIX, 483. Also, The basal metabolism of white rats in relation to the intake of vitamin B. *Proc. Amer. Physiol. Soc.*, *Amer. Jour. Physiol.*, 1924, LXVIII, 131.
4. Drummond, J. C., and Marrian, G. F., The physiological role of vitamin B. Part I. The relation of vitamin B to tissue oxidations. *Biochem. Jour.*, 1926, XX, 1229.

5. Kon, S. K., and Drummond, J. C., The physiological role of vitamin B. Part III. Study of vitamin B deficiency in pigeons. *Biochem. Jour.*, 1927, XXI, 632.
6. Rose, W. B., Stucky, C. J., and Cowgill, G. R., Gastric motility in vitamin-B deficiency. *Amer. Jour. Med. Sci.*, 1929, CLXXVII, 307. See also, Rose, W. B., and Stucky, C. J., Anhydremia in rats suffering from lack of what has been called vitamin B. *Proc. Soc. Exp. Biol. Med.*, 1928, XXV, 687.
7. Mitchell, H. H., and Carman, G. G., Does the addition of sodium chloride increase the value of a corn ration for growing animals? *Jour. Biol. Chem.*, 1926, LXVIII, 165.
8. Carroll, W. E., and Mitchell, H. H., Results of feeding copperas in paired-feeding experiments with growing swine. *Proc. Amer. Soc. An. Prod.*, Nov. 1927, 73.
9. Sherman, H. C., and Merrill, A. T., Cystine in the nutrition of the growing rat. *Jour. Biol. Chem.*, 1925, LXIII, 331.
10. Finks, A. J., Jones, D. B., and Johns, C. O., The role of cystine in the dietary properties of the proteins of the cow-pea, *Vigna sinensis*, and of the field pea, *Pisum sativum*. *Jour. Biol. Chem.*, 1922, LII, 403.
11. "Student," The probable error of a mean. *Biometrika*, 1908, VI, 1.
12. Mitchell, H. H., and Carman, G. G., The composition of the gains in weight and the utilization of food energy in growing rats. *Amer. Jour. Physiol.*, 1926, LXXVI, 398.

Note.—Since this article was written our attention has been called to the fact that the paired-feeding method has been used by W. E. Anderson and A. H. Smith (*J. Biol. Chem.* 1924, LXI, 181-191) in a study of the effect of acute scurvy on subsequent nutrition and growth of guinea pigs. "It was convincingly demonstrated by the carefully controlled methods employed that the loss of body weight observed in the present series of scorbutic guinea pigs at the height of scurvy cannot be accounted [for by the inanition factor alone. Growth in the postscorbutic stage is either parallel to or greater than that of the normal guinea pig and is accompanied by a definite increase of food intake."





# THE EFFECT OF INSULIN UPON THE BODY WEIGHT OF THE RABBIT

BY M. LOUISA LONG AND FRITZ BISCHOFF

(*From Potter Metabolic Clinic, Santa Barbara Cottage Hospital,  
Santa Barbara, Calif.*)

Received for Publication—July 17, 1929

## INTRODUCTION

SEVERAL diabetic patients of the Potter Metabolic Clinic, dietitians, and others interested in the treatment of diabetes, have felt that the use of insulin brings about an abnormal gain in weight. This idea, as far as we can gather, has not in any case been based upon a statistical survey. It would, indeed, be difficult to conceive of carrying out such a study on the diabetic patient. The severe diabetic, before taking insulin, is undernourished and underweight, and a gain in weight following insulin therapy is therefore to be expected. The mild diabetic, on the other hand, is often overweight. Joslin's studies (1) have shown rather conclusively that the incidence of diabetes is higher in the obese. When the severe diabetic on insulin treatment becomes obese he may be merely returning to his pre-diabetic weight. In the foreign literature (2, 3, 4, 5, 6) there are reports in which undernourished non-diabetic patients gained weight under the influence of insulin. The insulin is regarded as stimulating the appetite, so that a higher calorie intake of food is induced.

In this paper two pertinent questions concerned with the effect of insulin upon body weight are considered. 1. Does the administration of insulin to the normal animal, without the simultaneous augmentation of the caloric intake of food, bring about an increase in weight? 2. Does the administration of insulin to the normal animal stimulate the appetite, so that more food is eaten? Rabbits were used as experimental animals. In one series of experiments rabbits receiving doses of insulin and controls not receiving insulin were placed upon weighed diets. In the other series the rabbits receiving insulin and the controls were permitted to eat as much food as they desired, a record of the food intake and body weight being kept.

## EXPERIMENTAL

*Series 1.* Six rabbits, 4 males and 2 females, of the same litter, were started at 3 months of age on a weighed diet, consisting of 87.5 gms. alfalfa and 26 gms. of barley totaling approximately 250 calories daily. Three of

these rabbits, Nos. 3, 5, and 6, two males and one female, were given daily doses of 6 units of insulin subcutaneously. This was increased at the end of six weeks to 8 units. Rabbits Nos. 1, 2, and 4 served as controls. The intake of barley remained the same throughout the experiment; the alfalfa was increased after 5 weeks to 157 gms. per day, making the daily caloric intake 340, and decreased two weeks later to 120 gms., giving 280 calories per day.

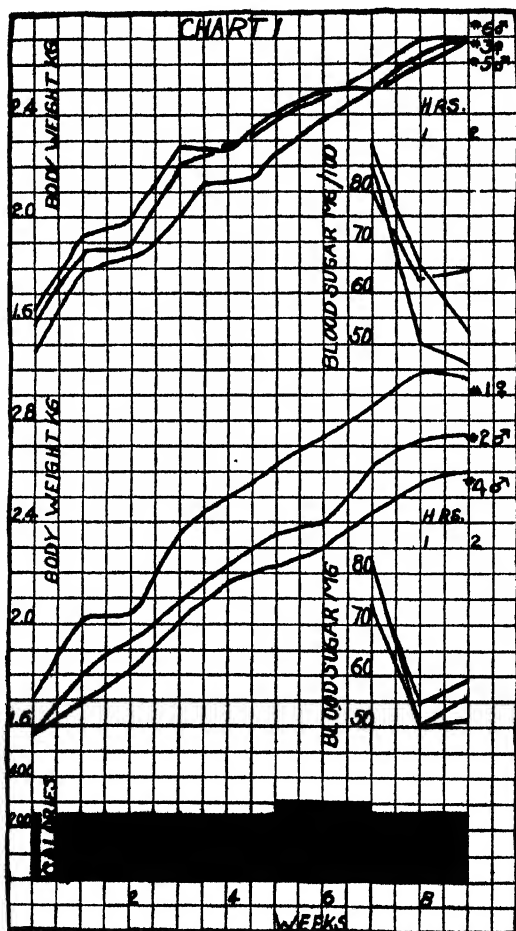


CHART 1.—Rabbits on restricted, weighed diet of barley and alfalfa.

Upper curves—growth and blood sugar curves of rabbits receiving daily subcutaneous doses of insulin.

Lower curves—growth and blood sugar curves of control rabbits.

The rabbits were weighed weekly. No differences in weight were observed between the rabbits receiving insulin and those not receiving it. The experiment was terminated at the end of ten weeks. At this time blood sugar

curves following insulin were determined for all of the rabbits on the experiment. This was done to be sure that a sufficiently large dose of insulin had been given to produce a distinct hypoglycemia.

*Series II.* In the second series the rabbits were given as much alfalfa as they would eat, and a weighed amount of barley each day. Six rabbits, four males and two females of the same litter, having an average weight of 1.7 kg., were started on the diet when 6 weeks old. Rabbits Nos. 7, 8,

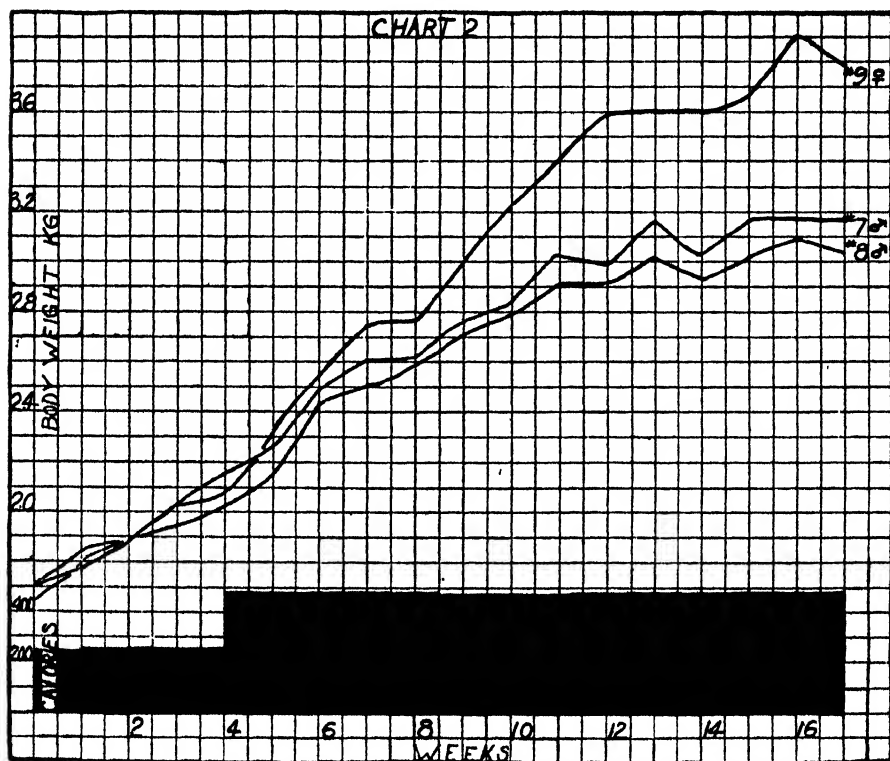


CHART 2.—Rabbits on an unrestricted diet of alfalfa and a weighed amount of barley.

Growth curves and caloric intake of rabbits in second series which received daily subcutaneous doses of insulin.

and 9, two males and a female, were given daily doses of insulin, beginning with 6 units and increasing by two units each two weeks until fourteen units were being given daily during the last month of the experiment. All were fed alfalfa *ad lib.*, a record being kept of the daily amount consumed during the first two months and a quantity in excess of this given during the remainder of the experiment. Twenty six grams of barley were given daily the first month and 52 grams thereafter.



The average daily intake of alfalfa for the rabbits taking insulin was 144.5 grams, and for the controls 152.8. An analysis of the barley and alfalfa was made in order to determine the caloric intake of the rabbits.

	Barley	Alfalfa
Protein.....	9.4%	16.7%
Fat.....	2.3%	1.8%
Carbohydrate.....	78.7%	17.7%
Calories per 100 gm.....	373.0	154.0

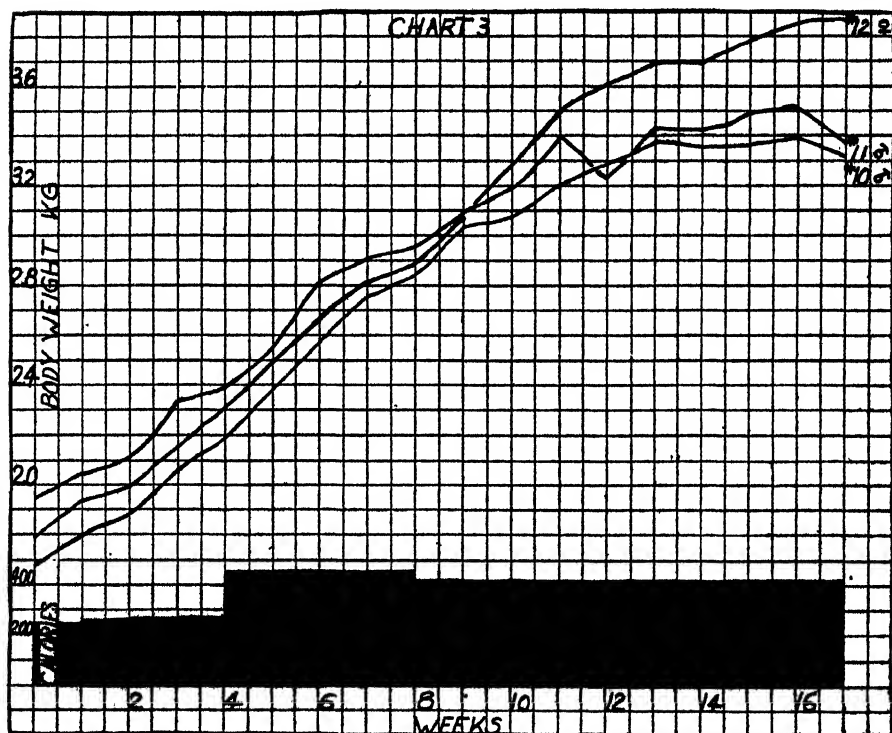


CHART 3.—Rabbits on an unrestricted diet of alfalfa and a weighed amount of barley. Growth curves and caloric intake of control rabbits in second series.

This would make their daily caloric intake as follows:

	Alfalfa	Barley	Total
Insulin rabbits, 1st mo.....	223	97	320
Remainder of experiment.....	223	194	417
Controls, 1st mo.....	235	97	332
Remainder of experiment.....	235	194	429

As can be seen from the growth curves, the increase in weight was practically the same for the two groups of rabbits.

## CONCLUSION

Feeding experiments started upon young rabbits and carried through to maturity show that insulin does not bring about increased gain in weight either when the calorie intake is restricted, or when the animals are permitted to eat as much as they desire. Since the animals were not diabetic, no definite conclusion can be made in regard to the diabetic patient. It would be impossible to settle the question upon depancreatized animals because of the digestive disorders following the removal of the pancreas. It would also be impossible to establish the pre-diabetic weight of a patient. One is, therefore, tempted to draw a parallelism between the normal rabbit and the human diabetic, and to say that insulin does not cause obesity in the diabetic, but merely brings him back to his pre-diabetic weight.

## BIBLIOGRAPHY

1. Joslin, Elliot P., *Jour. Amer. Med. Assoc.*, 1921, LXVI, 79.
2. Bauer, R., and Nijiri, *Medizinsche Klinik*, 1925, XXI, 1454.
3. Bauer, R., *Klinische Wochenschrift*, 1928, VII, 1743.
4. Böckheler, T., *Münchener Medizinische Wochenschrift*, 1926, LXXIII, 1921.
5. Fonseca, F., *Archives für Verdauungs Krankheiten*, 1928, XLII, 362.
6. Haemmerli, A., *Schweizerische Medizinische Wochenschrift*, 1926, LVI, 1095.





## VITAMIN STUDIES XVII. OSSIFYING POTENCY OF RAW AND EVAPORATED MILKS\*

BY HANNAH E. HONEYWELL, R. ADAMS DUTCHER AND CHESTER D. DAHLE

*(From the Department of Agricultural Biochemistry, Pennsylvania  
State College, State College, Pa.)*

Received for Publication—July 18, 1929

THE investigations described in this paper are a continuation of our work on the vitamin content of raw and evaporated milk. Previous papers have dealt with the amount of vitamin A and of the B complex found in the milk of the Pennsylvania State College herd, and in the evaporated milk made therefrom. The present data represent an attempt to evaluate the ossifying potency of whole and treated milk.

In regard to the antirachitic potency of milk, published data exhibit conflicting results. Steenbock, Hart, Hoppert and Black (1), using their yellow corn ration (No. 2965), found that 12 cc. (daily) of cows' milk were the minimum requirement for the healing of rickets in rats. Luce (2) reported that, 2 to 5 cc. of milk from pasture-fed cows would prevent rickets in rats fed on the McCollum ration (No. 3143), but that 15 to 20 cc. of milk from cows kept in the dark were ineffective. Hess and Weinstock (3) stated that, in an experiment of the preventive type, 20 to 25 cc. of milk prevented rickets. Outhouse, Macy and Brekke (4), using the Osborne and Mendel rachitogenic ration, and a curative technique, found that "30 cc. of cows' milk fed daily for 7 days induced marked healing of rachitic lesions in rats." Differences in the environment and feeding of the cows as well as in rations, in animals, and in the experimental technique, make explanation of these conflicting results difficult.

In the present experiments the milk came from the college certified herd which is kept under conditions carefully standardized as to rations, exercise, and light. As described in our previous papers (5, 6), the evaporated milks were made from the same sample as the raw milk fed, and under conditions approximating those used in commercial practice. In this way the evaporated milks could be tested before and after sterilization. From June until January five kinds of milk, raw (RM), vacuum evaporated (VE), vacuum-evaporated-sterilized (VES), air-evaporated (AE), and air-evap-

\* Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as technical paper No. 480.

orated-sterilized (AES), were fed. Before feeding, the evaporated milks were diluted to the concentration of the raw milks in order to compare them with equal volumes of the raw certified milk from which they were made.

The animals were chosen carefully from the "Penn State strain" of pied rats reared in this laboratory on a diet the constituents of which have not been varied for several years (7). Each animal was placed in an individual cage and given the Steenbock yellow corn ration (No. 2965) (8) for 21 days, with the prescribed volume of milk in separate doses daily. Lighting conditions were uniform throughout, and no sunshine, even through glass, was allowed to fall on the cages. Equal numbers of male and female rats, 21 days of age and weighing between 36 and 42 grams, were evenly distributed throughout the experiment. At this age the amount of ash in the femurs of our experimental animals averaged about 42 per cent. At the end of the 21-day feeding period, the rats were killed with ether and examined carefully for beading of ribs, enlargement of joints, and softness of bones. The right humerus was preserved for the line test of McCollum and co-workers (9), and the femurs were removed for ashing according to our previous technique (7). The bones were dried at constant temperature in the electric oven for 24 hours, extracted with 95 per cent alcohol, and again with ether for the same length of time, dried, and ashed in a muffle furnace at about 600° C. On the basis of the dry extracted bone, the percentage of ash and the ratio of ash to organic matter (A/R) were calculated.

The data are summarized in Table I. The bone-ash recorded represents group averages and when distinct groups were fed the same quantity of milk at different seasons, the averages are given separately and, later, together. The raw milk was fed in 5, 10, 12, 15, and 20 cc. portions. The 5 and 10 cc. doses produced a bone-ash of 35 and 39 per cent, respectively, and both were below the original 42 per cent level of the bone-ash in the femurs at the time when the animals were placed on the experimental diet. The addition of 12 cc. of raw milk raised the bone-ash to 43.4 per cent, which is higher than the original level and yet not so high as the ash (53 per cent) produced in animals of the same age which have been fed on the stock ration for the same length of time. Since 5 and 10 cc. of raw milk (daily) did not increase the bone-ash and, since the ingestion of 20 cc. was very difficult for such small animals during the first part of the feeding period, 10, 12 and 15 cc. quantities were chosen as the levels at which the evaporated milks were to be fed.

The milks were fed from June, 1926, to January, 1927, and during July,

1927, the experiment was continued as before with the exception that equal amounts of milk-ash were fed to ascertain the contribution made by the minerals alone. The seasonal variations in ossification were not marked in animals receiving 10 cc. except in the group receiving raw milk, which may have been due to the feeding of small amounts of green feed to the college herd in June, the month in which one of the two determinations on raw milk was made. On the 12 cc. level seasonal variations were evident, in all the experimental groups in which two seasons were represented, and since in each case July was one of the months represented in the group, the ration given to the cows may account for the difference in the ossifying potency of the milk. This coincides with the experience of Luce (2) who found greater "antiricketic value" in milk from the same cow when she was pasture fed than when eating a dry ration.

The experimental milks were fed at the 10 cc. level and the percentages of bone-ash obtained were as follows: RM 38.3, VE 33.4, VES 27.8, AE 29.5, AES 25.5. When 12 cc. were fed, the percentages of bone-ash were RM 43.4, VE 39.3, VES 38.1, AE 35.0, AES 34.8. At the 15 cc. level, the percentages of bone ash produced were RM and VE 44.8, VES 44.1, AE 42.5, and AES 40.3. The differences between the milks are less marked at this level, possibly because, with this quantity, both the minerals and the calcifying potency of the milk are increased to the point where deposition is equal or superior to loss of bone salts.

According to Daniels and Loughlin (10), some of the differences between the evaporated and raw milks may be due to the mechanical removal of a portion of the mineral salts from the milk by the evaporation process. Evaporated milks made from the same original sample by the vacuum method and by the air-evaporation method, when fed in the same amounts produce a distinct and different lowering of the bone-ash. A difference in the method of evaporation might produce a difference in the mineral content and, therefore, a difference in the amount of ash in the bones of animals to which the two types of milk were fed, but when especial care was taken to prevent the loss of minerals by the use of glass containers, sterilization again produced a lowering in the bone-ash. Therefore, some of the variations in the bone-ash may have been due to loss of antirachitic potency as well as to direct loss of minerals in the evaporation process.

The bone-ash of the animals fed 20 cc. of raw milk averaged 48.8 per cent. This is nearly a normal value and agrees with the statement of Krauss (11), using 0.8 gm. of butter fat, that 23 cc. milk are required for normal bone formation in rats fed a rachitic ration. Our data on 20 cc. of raw milk and on evaporated milks are not in agreement with Daniels

and Jordan (12), but, owing to lack of detailed data in their preliminary report and the apparent differences in technique, we are unable to explain the discrepancies at the present writing. Furthermore, the amount of antirachitic factor in our milks must have been much less than that observed by Daniels and Jordan, since they irradiated their milk samples with ultra violet light. It has been our experience that it is often necessary to feed a vitamin at the "threshold" level in order to note, with certainty, its susceptibility to heat treatment.

To determine the effect of the minerals alone on the rate of ossification, samples of raw, vacuum-evaporated, and vacuum-evaporated-sterilized milks were ashed. The ash was dissolved in dilute hydrochloric acid and made up with distilled water to the original volume of the milk from which the ash was prepared. One group of rats were fed 12 cc. aliquots, while their litter mates received the same volume of the original samples of milk. Unfortunately, at this time, air-evaporated milk samples were not available, since the evaporator had been removed from the dairy plant on the assumption that no more milk would be needed for this experiment.

The results of the feeding of the milk ash are shown in Table I. The bone of the rats which received ash from raw milk averaged 36.3 per cent, 9 per cent lower than their litter mates which were fed the same volume of raw milk. The separate groups of animals which received the two evaporated milks yielded approximately the same bone-ash, 33.8 and 33.7 per cent. The difference between the results given by the vacuum-evaporated milk and the same milk sterilized is practically negligible. The three groups which received milk ash produced a bone-ash which does not differ materially from that obtained with the air-evaporated milks, sterilized and unsterilized. These are the samples on which the greatest amount of injury was noted.

According to Chick and Roscoe (13), "The larger variations in bone composition seem to be confined to the water, fat, and mineral constituents. It seems probable that the value of the ratio, mineral ash: organic residue, might after all be the most trustworthy guide to the efficiency of the process of calcification." Therefore, the A/R ratios were calculated and are given in the table. Compared with the percentage of bone-ash they show the same general trend, *i.e.*, as the amount of bone-ash grows larger, the A/R ratio increases.

The line tests showed that the wide and irregular bands of cartilage had little or no deposition of calcium salts in the provisional zone of calcification at their base. As the cartilage bands became narrower and straighter, the line at the bases became heavier, showing that calcium salts had been

TABLE I

Milk	cc.	Month	No. of animals	Bone Ash per cent	A/R	Mean		Line test cartilage band
						Ash	A/R	
<i>RM</i>	5	June	8	35.2	.542	35.2	.542	
	10	June	7	40.0	.667	38.3	.622	Wide—fairly regular
		Dec.	8	36.9	.584			
	12	Dec.	12	41.7	.716	43.4	.775	Wider than normal bone—regular
<i>(RM Ash)</i>		July	10	45.5	.846			
	12	July	10	36.3	.572	36.3	.572	Wide—irregular
	15	Aug.	10	44.9	.817	44.8	.812	Narrower than 12 cc. RM—regular
		Oct.	4	44.6	.805			
	20	Aug.	8	48.8	.953	48.8	.953	Nearly like normal bone—regular
<i>VE</i>	10	Sept.	9	34.2	.521	33.4	.502	Wide—irregular
		Dec.	6	32.2	.475			
	12	Jan.	10	36.9	.586	39.3	.651	Wider than 12 cc. RM—regular
		July	10	41.7	.716			
<i>(VE Ash)</i>	12	July	10	33.8	.513	33.8	.513	Wide—irregular
	15	Oct.	9	44.8	.812	44.8	.812	Wider than 15 cc. RM—regular
<i>VES</i>	10	Sept.	10	27.7	.384	27.8	.388	Very wide—irregular
		Dec.	6	27.9	.395			
	12	Jan.	10	35.4	.548	38.1	.618	Wider than 12 cc. VE—irregular
		July	10	40.7	.688			
<i>(VES Ash)</i>	12	July	10	33.7	.511	33.7	.511	Very wide—irregular
	15	Oct.	10	44.1	.778	44.1	.778	Wider than 15 cc. VE—fairly regular
<i>AE</i>	10	Sept.	10	29.3	.414	29.5	.420	Very wide—irregular
		Dec.	6	29.8	.432			
	12	Jan.	10	35.0	.540	35.0	.540	Wider than 12 cc. RM—irregular
	15	Jan.	10	42.5	.743	42.5	.743	Wider than 15 cc. RM—regular
<i>AES</i>	10	Sept.	9	25.4	.340	25.5	.344	Very wide—irregular
		Dec.	5	25.8	.351			
	12	Jan.	10	34.8	.534	34.8	.534	Wider than 12 cc. AE—irregular
	15	Jan.	10	40.3	.673	40.3	.673	Wider than 15 cc. AE—fairly regular
Breeding ration						52.9	1.12	Narrow band—very regular



laid down. This band became narrower and more regular as the A/R ratio and the percentage of the bone-ash increased.

### SUMMARY

Prophylactic experiments are described in which milk was added to a rachitic diet. Evaporated milks made by vacuum and aeration methods were fed at different levels in direct comparison with the raw milk from which they were made. A slight seasonal variation is shown in both the raw and evaporated milks.

The ossifying potency of the treated milks is less than that of the raw. Vacuum evaporation, aeration, and sterilization tended to decrease the ossifying potency of raw milk.

Ash, equivalent to 12 cc. of raw, vacuum-evaporated, and vacuum-evaporated-sterilized milk, was fed in comparison with the original samples. In all cases the milk-ash showed less ossifying potency than the milks from which they were obtained, indicating that a part of the calcifying properties of the various milk samples must have been due to the presence of vitamin D.

The data obtained indicate that the milks described are not rich in vitamin D. While commercial methods of evaporation and sterilization evidently affect the ossifying potency of milk to an appreciable degree, no attempt has been made to differentiate between mineral loss and vitamin D destruction.

### BIBLIOGRAPHY

1. Steenbock, H., Hart, E. B., Hoppert, C. A., and Black, A., *Jour. Biol. Chem.*, 1925, LXVI, 441.
2. Luce, E., *Biochem. Jour.*, 1924, XVIII, 716.
3. Heas, A. F., and Weinstock, M., *Amer. Jour. Dis. Child.*, 1927, XXXIV, 845.
4. Outhouse, J., Macy, I., and Brekke, V., *Jour. Biol. Chem.*, 1928, LXXVIII, 129.
5. Dutcher, R. A., Honeywell, H. E., and Dahle, C. D., *Jour. Biol. Chem.*, 1927, LXXV, 85.
6. Dutcher, R. A., Francis, E., and Combs, W. E., *Jour. Dairy Sci.*, 1926, IX, 379.
7. Dutcher, R. A., Creighton, M., and Rothrock, H. A., *Jour. Biol. Chem.*, 1925, LXVI, 401.
8. Steenbock, H., and Black, A., *Jour. Biol. Chem.*, 1925, LXIV, 263.
9. McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *Jour. Biol. Chem.*, 1922, LI, 41.
10. Daniels, A., and Loughlin, R., *Jour. Biol. Chem.*, 1920, XLIV, 381.
11. Krauss, W. E., *Bull. Ohio Expt. Sta.*, 1929, XIV, 57.
12. Daniels, A., and Jordan, D., *Proc. Soc. Exp. Biol. and Med.*, 1929, XXVI, 453.
13. Chick, H., and Roscoe, M. H., *Biochem. Jour.*, 1926, XX, 137.



## THE RELATION OF THE FAT-SOLUBLE VITAMINS (A AND D) TO THE DEVELOPMENT OF EX- PERIMENTAL RICKETS IN RABBITS

BY ALAN R. MORITZ AND CARL KRENZ\*

*(From the Department of Pathology, School of Medicine,  
Western Reserve University, Cleveland, Ohio.)*

Received for Publication—July 19, 1929

**E**XPERIMENTAL results have been at some variance as regards the etiological interdependence of the fat-soluble vitamins with calcium and phosphorus in rickets. The production of rickets in puppies by a diet deficient in fat-soluble vitamins (Mellanby) followed by a demonstration of the relationship of this factor to calcium and phosphorus metabolism (McCollum, Simmonds, Shipley and Park; Sherman and Pappenheimer; Korenchevsky), has stimulated attempts to define the relative importance of these factors in the production of experimental rickets.

Mellanby concluded that rickets developed in puppies whose diets were deficient only in the antirachitic fat-soluble organic factor. Attempts to produce rickets in guinea pigs (Tozer), kittens (Mackay), and pigs (Zilva, Golding, Drummond and Coward), by feeding a diet deficient only in the fat-soluble vitamins, failed. Korenchevsky and also Goldblatt produced rickets in rats by a diet deficient only in the fat-soluble vitamins, although Shipley, Park, McCollum and Simmonds, and Hess, McCann and Pappenheimer concluded that there must be a deficiency both of the fat-soluble vitamin and of calcium or phosphorus to produce rickets in rats.

Rickets has been produced in the rabbit by diets deficient in fat-soluble vitamin, low in phosphorus, and high in calcium (Goldblatt and Moritz). It was considered of interest then to observe the effects of a diet deficient only in the fat-soluble vitamins (A and D) on the degree of calcification of the bones and the development of rickets in rabbits and at the same time to repeat the experiment on rats.

### EXPERIMENTAL

*Diet.* The basal diet used was essentially the same as that used for the production of rickets in rats by Korenchevsky and by Goldblatt. This diet was eaten readily by the rabbits; only 4 of the 30 observed refused to eat.

Each animal received daily 5 grams of alcohol- and ether- extracted

\* Hanna Research Fellow.

and dried alfalfa hay (Goldblatt and Moritz) to supply roughage, and 7.5 cc. of decitrated lemon juice to supply the antiscorbutic factor.

The animals were divided into four groups as follows. Group I received a normal diet consisting of milk, oats, lettuce, cabbage, and alfalfa hay. This diet will be referred to as (N); Group II received a complete diet consisting of the basal diet in which the salt mixture used was McCollum's No. 185. This diet contained 0.6 per cent of Ca (determined) and 0.8 per cent of phosphorus (calculated). With a daily intake of 5 grams of alfalfa hay containing 2.14 per cent of calcium (Forbes) an adequate calcium intake was assured. Each animal received 10 drops (about 220 milligrams) of potent cod liver oil daily. This diet will be referred to as (-A+CLO). Group III received a complete diet except for fat-soluble vitamin, and was the same as diet (-A+CLO), but without the cod liver oil. This will be referred to as diet (-A). Group IV received diet (-A-P), deficient in phosphorus and fat-soluble vitamin, and consisted of the basal diet in which a salt mixture devoid of phosphorus (No. 6 of Korenchevsky) was used.

*Animals.* Litters of young rabbits varying in age from 33 to 47 days were used. Each litter was divided in such a manner that comparable animals would be in each dietetic group. Four were placed in group I, eleven in group II, eleven in group III and two in group IV.

All animals were weighed twice a week. The amount of food consumed daily by each animal was noted. Two animals belonging to the same group were kept in each cage in a room receiving no direct sunlight.

After varying periods of observation on the diet, comparable animals were selected from each group and killed by bleeding from the carotid artery.

*Blood Chemistry.* The determination of calcium by the Tisdall modification of the Kramer-Tisdall method and of inorganic phosphorus by the Tisdall method were made on the blood serum obtained at the time of death.

*Bone Analysis.* The right and left radii removed at the time of autopsy were carefully cleaned of all adherent soft tissue and weighed separately. The bones were then dried for 12 hours at 110° C, weighed again and ashed. The ash was dissolved in 2 cc. of concentrated hydrochloric acid, made up to a volume of about 75 cc. with distilled water in a 100 cc. volumetric flask and neutralized with ammonium hydroxide. Acidification was then brought about with an excess of 5 drops of concentrated hydrochloric acid, water was added to bring the volume up to 100 cc. and this solution thoroughly mixed.

Aliquots of 2 cc. were then pipetted into each of two narrow tipped 15 cc. centrifuge tubes to which were added 1 cc. of a 20 per cent solution of sodium acetate and 1 cc. of a saturated solution of ammonium oxalate. After standing for one hour the calcium oxalate was separated by centrifuging 15 minutes at high speed and washed twice with 2 per cent ammonium hydroxide, as in the Kramer-Trisdall method for serum calcium.

The washed calcium oxalate was dissolved in 3 cc. of N/1 sulphuric acid and titrated against potassium permanganate which had been standardized against N/50 sodium oxalate.

Two determinations of calcium were thus made on each bone, and from these figures and those of the bones, before and after drying, the percentages of water and of calcium per wet and dry weight of bone were calculated.

*Histological Examination.* The sixth, seventh and eighth ribs from the right side were fixed in formalin, decalcified in Muller's fluid and stained with hematoxylin and eosin.

*X-Ray Examination.* X-ray photographs were taken once a week on animals 1386, 1387, 1391, 1393 and 1400.

All observations and data on these animals, except the growth curves and x-rays are recorded in Table I. There is a definite reduction of the serum calcium of the rabbits in group III, on (-A) diet, while there are no significant differences in the amounts of serum calcium in the other three groups. The inorganic serum phosphorus of rabbits in group IV is greatly reduced but is normal in the other three groups.

In groups I, II and III there are no consistent differences in the percentages of calcium in the bones. However, the average percentage of calcium in the bones of group III is decreased per wet weight as compared with those in groups I and II. The figures show that the amount of calcium per whole bone is reduced in group III, but the number of observations is too small to determine, on the basis of statistical analysis, that the diminution in the average percentage of calcium per net weight represents a real decrease in the degree of calcification of the bones of rabbits on diet (-A).

The rabbits in group IV show a definite reduction in the percentage of calcium per wet and dry weights of the bones.

Both gross and histological examination of the bones of the animals in group I showed them to be normal, while those of group II were normal or slightly osteoporotic. There was neither gross nor histological evidence of rickets in any of the animals of group III, although these animals were killed and examined after having been on the diet from 14 to 43 days. There were varying degrees of osteoporosis. At the end of the

TABLE I

Rabbit No.	Initial Age in Days	Days on Diet	Weight in grams		Blood mgm. per cent		Bones	
			Initial	Final	Ca	P	Ca Per cent Wet wt.	Micro. Exam.
Group I (Normal Diet)								
1499	49	16	1135	1340	17.25	8.76	11.86	Normal
1498	64	24	1312	1460	17.81	8.00	13.43	Normal
1500 A	50	24	867	1075	15.49	5.63	10.47	Normal
1495	41	33	612	1170	16.21	9.15	10.75	Normal
(Averages)					—	—	—	
Group II (-A+CLO) Diet)								
1395	40	31	360	625	12.69	8.94	8.36	Normal
1409	33	31	660	885	16.89	6.41	9.26	Normal
1496	39	35	562	920	13.56	10.61	9.53	Osteoporosis
1497	39	35	597	1020	16.32	8.70	9.48	Normal
1408	33	40	579	1115	16.81	8.42	11.82	Normal
1392	43	41	425	830	12.74	9.09	9.79	Normal
1397	37	41	477	1050	14.58	8.10	11.35	Osteoporosis
1504	47	42	770	1270	12.46	8.27	9.96	Osteoporosis
1505	47	42	780	1360	15.08	7.45	11.66	Osteoporosis
1393	41	45	652	1265	15.09	8.78	11.85	Normal
1394	41	45	575	1297	16.98	8.42	12.00	Normal
(Averages)					14.83	8.47	10.46	
Group III (-A) Diet)								
1500	47	14	870	735	10.75	8.76	7.86	Osteoporosis
1389	40	31	379	485			8.08	Osteoporosis
1390	40	31	404	540	10.15	8.85	6.81	Normal
1410	33	32	632	810	14.35	6.48	10.27	Normal
1502	47	32	815	995	8.03	8.79	10.02	Normal
1493	39	35	643	625	7.48	9.15	7.50	Osteoporosis
1391	37	41	472	635	8.04	9.39	8.04	Osteoporosis
1386	43	41	447	580	9.49	5.46	8.04	Osteoporosis
1503	47	42	750	780	8.24	6.79	8.41	Osteoporosis
1501	47	42	680	830	8.24	9.37	9.65	Osteoporosis
1387	41	43	617	713			10.35	Osteoporosis
(Averages)					9.41	8.11	8.63	
Group IV (-A-P) Diet)								
1400	37	45	574	955	16.86	4.34	7.47	Rickets
1399	41	47	609	1020	15.43	2.75	7.23	Rickets

experimental period some of these animals were still gaining weight, some had stopped gaining and others were on the decline. The animals in group IV showed moderate rickets.

Chart 1 contrasts the composite curves of the weekly weight increases of the rabbits in group II and III.

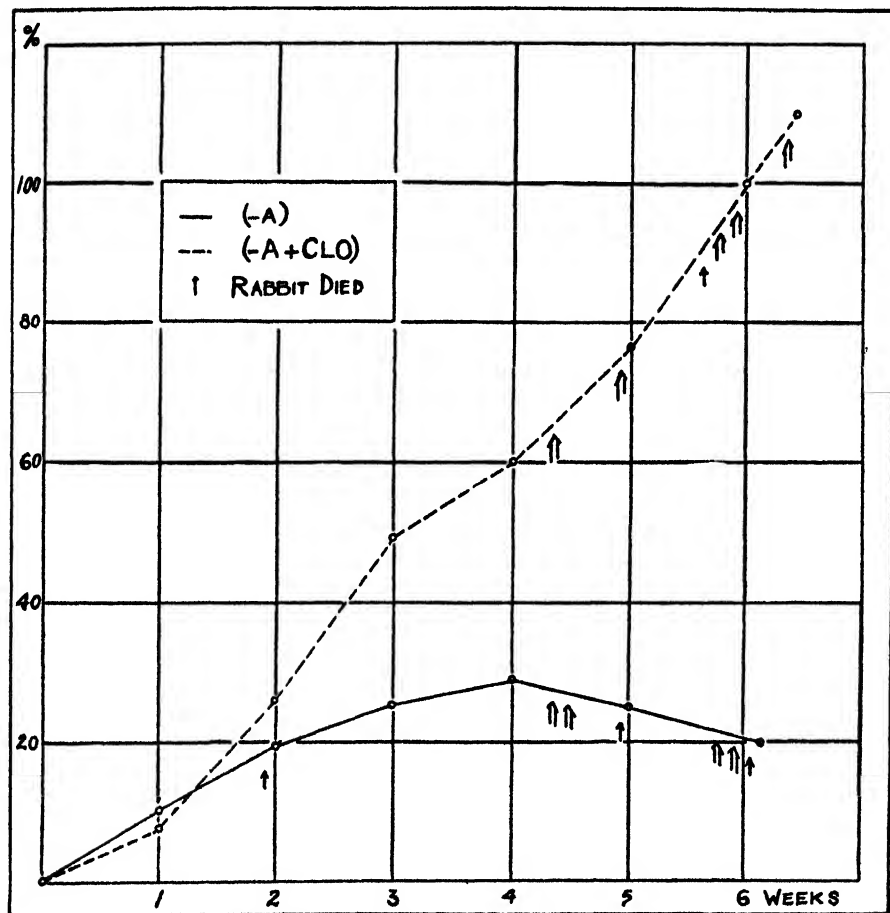


CHART 1.—Composite curves of percentage weight increase in eleven rabbits on diet (-A) and eleven on diet (-A+CLO).

Weekly x-ray photographs of rabbits 1386, 1387 and 1391 on diet (-A) showed no evidence of rickets nor did rabbits 1392 and 1393 on diet (-A+CLO). Rabbit 1400 on diet (-A-P) showed distinct changes at the end of the second week indicative of rickets which became progressively more marked and consisted in the development of a rachitic metaphysis separating the epiphysis and diaphysis seen at the distal end of the

radius, ulna, humerus, tibia, fibula and femur. There was cupping and imperfect calcification of the diaphysis as well as deficient calcification of the epiphysis.

#### EXPERIMENTS ON RATS.

As a dietary control 9 rats were placed on the same diets fed the rabbits in the following manner: 3 on diet (-A+CLO); 3 on diet (-A); 2 on diet (-A-P) and one on a diet deficient in the fat-soluble vitamin and calcium, referred to as diet (-A-Ca). In this diet a salt mixture devoid of calcium (No. 2 of Korenchevsky) was used. Five parts of decitrated lemon juice were added directly to the diet (except in diet (-A-Ca) in which whole lemon juice was used) and alfalfa was omitted.

TABLE II

Rat Number	Initial age in Days	Days on Diet	Weight in Grams		Bones	
			Initial	Final	Ca per cent Wet wt.	Micro. Exam.
Group I ((-A+CLO) Diet)						
1417	38	45	46	138	14.24	Normal
1418	38	45	63	122	13.49	Normal
1419	30	45	23	70	10.95	Normal
Group II ((-A) Diet)						
1414	38	44	46	82	10.53	Osteoporosis
1415	38	44	71	117	11.36	Osteoporosis
1416	30	44	38	70	7.92	Rickets
Group III ((-A-P) Diet)						
1420	30	37	42	83	8.19	Osteoporosis
1421	30	37	36	64	5.56	Rickets
Group IV ((-A-Ca) Diet)						
1422	30	36	42	62	6.65	Rickets

Calcium analysis was made on femur, fibula and tibia from both legs of each animal and histological examination was made on the ribs.

Table II shows that the percentage of calcium in wet bones of rats on diet (-A) was distinctly lower than in those on diet (-A+CLO) although not so low as in the rats on diet (-A-P) or (-A-Ca).

Histological examination showed the bones of rats on diet ( $-A + CLO$ ) to be normal. There was definite rickets in rat 1416 on diet ( $-A$ ) while the other two showed only osteoporosis. The percentage of calcium in both wet and dry bones of rat 1416 was markedly decreased. Of the two rats on diet ( $-A - P$ ), one showed severe rickets and the other severe osteoporosis. The rat on diet ( $-A - Ca$ ) showed rickets.

### CONCLUSIONS

1. Rabbits fed on a diet deficient only in the fat-soluble vitamins (diet  $-A$ ) showed no evidence of rickets at the end of from 14 to 45 days.
2. The blood serum calcium was almost uniformly decreased.
3. The chemical examination of the bones of the rabbits suggests that the amount of calcium in the whole bone was reduced. In the three rats on the diet deficient in fat-soluble vitamins (diet  $-A$ ) the amount of calcium in the bone was unquestionably reduced and rickets occurred in one of the animals.

The authors wish to acknowledge their thanks to Dr. Harry Goldblatt for his helpful criticisms and suggestions.

### BIBLIOGRAPHY

- Forbes, E. B., *Bull. Ohio Exper. Station*, 207, (cited by McCollum, 1917).
- Goldblatt, H., A study of the relation of the quantity of fat-soluble organic factor in the diet to the degree of calcification of the bones and the development of experimental rickets in rats. *Biochem. Jour.*, 1923, XVII, 298.
- Goldblatt, H., and Moritz, A. R., Experimental rickets in rabbits. *Jour. Exper. Med.*, 1925, XLII, 499.
- Hess, A. F., McCann, G. F., and Pappenheimer, A. M., Experimental rickets in rats. II. The failure of rats to develop rickets on a diet deficient in vitamin A. *Jour. Biol. Chem.*, 1921, XLVII, 395.
- Korenchevsky, V., Experimental rickets in rats. *Brit. Med. Jour.*, 1921, II, 547.
- Korenchevsky, V., The aetiology and pathology of rickets from an experimental point of view. *Med. Res. Council, Spec. Rep. Series*, No. 71, 1922, London.
- McCollum, E. V., The supplementary dietary relationships among our natural food stuffs. *Jour. Amer. Med. Assoc.*, 1917, LXVIII, 1379.
- McCollum, E. V., Simmonds, N., Shipley, P. G., and Parks, E. A., Studies on experimental rickets. I. The production of rachitis and similar diseases in the rat by deficient diets. *Jour. Biol. Chem.*, 1921, XLV, 333.
- Mackay, H. M. M., III. The effect on kittens of a diet deficient in animal fat. *Biochem. Jour.*, 1921, XV, 19.
- Mellanby, E., The part played by an accessory factor in the production of experimental rickets. (Proc. Physiol. Soc., Jan. 26, 1918.) *Jour. Physiol.*, 1918, LII, 11.
- Mellanby, E., Experimental rickets. *Med. Res. Council Spec. Rep. Series* No. 61, London, 1921.
- Sherman, H. C., and Pappenheimer, A. M., A dietetic production of rickets in rats and its prevention by an inorganic salt. *Proc. Soc. Exper. Biol. and Med.*, 1920-21, XVIII, 267.
- Shipley, P. G., Park, E. A., McCollum, E. V., and Simmonds, N., Studies on experimental rickets. III. A pathological condition bearing fundamental resemblances to rickets in the



- human being resulting from diets low in phosphorus and fat-soluble A; the phosphate ion in its prevention. *Johns Hopkins Hosp. Bull.*, 1921, XXXII, 160.
- Tisdall, F. F., A rapid colorimetric method for the quantitative determination of the inorganic phosphorus in small amounts of serum. *Jour. Biol. Chem.*, 1922, L, 329.
- Tisdall, F. F., A note on the Kramer-Tisdall method for the determination of calcium in small amounts of serum. *Jour. Biol. Chem.*, 1923, LVI, 439.
- Tozer, F. M., The effect on the guinea pig of deprivation of vitamine A and of the antiscorbutic factor with special reference to the conditions of the costo-chondral junctions of the ribs *Jour. Path. and Bact.*, 1921, XXIV, 306.
- Zilva, S. S., Golding, J., Drummond, J. C., and Coward, K. H., XLIX. The relation of the fat-soluble factor to rickets and growth in pigs. *Biochem. Jour.*, 1921, XV, 427



## THE VITAMIN A CONTENT OF YELLOW AND WHITE-CAPPED YELLOW DENT CORN\*

BY WALTER C. RUSSELL

*(From the Department of Agricultural Biochemistry,  
New Jersey Agricultural Experiment Station,  
New Brunswick, New Jersey.)*

Received for Publication—July 22, 1929

IN 1919, Steenbock (1) called attention to the association between certain yellow pigments and vitamin A in nature and formulated a working hypothesis which made the assumption that "the fat-soluble vitamin is a yellow plant pigment or closely related compound." A short time before this, Drummond (2) had reported vitamin A to be absent from crystalline carotin (carotene) but that a crude carotin fraction contained slight traces of the vitamin. Later, Rosenheim and Drummond (3) showed that fat-soluble A is not identical with carotin or xanthophyll. Also, Stephenson (4) found that carotin does not have the properties of vitamin A. More recently Widmark (5) has reported that plants which lose ability to form chlorophyll or lipochrome also are unable to form vitamin A. In 1928, v. Euler, v. Euler, and Hellström (20) presented data which indicate that carotin preparations have a high vitamin A potency, although their contention is disputed by Duliere, Morton and Drummond (21), and a recent report (1929) by Collison, Hume, Smedley-MacLean and Smith shows that carotin from cabbage fat has high vitamin A potency (22).

Soon after the suggestion of the above relationship by Steenbock, Palmer (6) called attention to exceptions to the hypothesis, the basis for the exceptions being chiefly the results of his investigations (7) and that of himself and Kempster (8) on the relation of plant carotinoids to the animal organism. Also Drummond and Coward (9) failed to find the association noted by Steenbock to prevail in all cases.

A study of a variety of substances by Steenbock and his associates (10, 11, 12, 13, 14,) confirmed the general relationship between the yellow pigments and the presence of vitamin A but they noted that the amount of vitamin does not always parallel the pigment content. The suggestion was made (1, 12) that possibly the pigment is in a leuco form in cases in which the vitamin content is high and the pigmentation slight. Such a

\* Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.

suggestion would apply in the case of the colorless fats noted by Drummond and Coward (9) and Palmer, Kennedy and Kempster (15). The latter authors review and discuss critically the data and the hypothesis concerning the relationship of yellow pigments to vitamin A.

Of particular interest in the present report is the comparison of the vitamin A content of white and yellow maizes by Steenbock and Boutwell (10), who showed the vitamin to be present in the yellow variety but not in demonstrable amounts in the white. Also, numerous practical feeding trials have demonstrated the superiority of the yellow variety as a source of vitamin A.

In view of the local production of white-capped, yellow dent corn it was of interest to determine quantitatively the amount of vitamin A in this variety as compared with that of the yellow variety, for the information of the stock feeder. There was also in mind the possibility that the data might be an addition to that already existing on the relation of vitamin A to pigments.

#### EXPERIMENTAL PROCEDURE

The procedure for the determination of vitamin A was essentially that outlined by Sherman and Munsell (16) except where otherwise indicated. The breeding colony ration was Sherman's No. 13, consisting of 2 parts ground whole wheat, 1 part whole milk powder and 2 per cent of the weight of the wheat as sodium chloride (17), modified by the incorporation of 10 per cent meat scrap (Swift's) at the proportionate expense of the wheat and milk. Young white rats, weaned at 28 days of age and weighing between 35 and 55 gm., were placed upon a basal, vitamin A-free diet, which was essentially that described by Sherman and Munsell, Diet No. 380, but modified to include the antirachitic factor. It was composed of purified casein 20 per cent, Osborne and Mendel (18) salt mixture 4 per cent, sodium chloride 1 per cent, dried yeast 10 per cent, corn starch 63 per cent, and 2 per cent of olive oil, containing 25 mg. cholesterol per cc., the oil and dissolved cholesterol having been irradiated with a quartz mercury-vapor lamp for 30 minutes at 60 cm. The vitamin B complex was determined in the yeast and if sufficiently potent the amount was decreased to 5 per cent and the corn starch increased correspondingly.

During the pre-test period the animals were kept in groups but during the test period they were confined in individual cages. After transfer to the individual cages, yeast and the olive oil solution of cholesterol were removed from the basal diet and fed as separate supplements, 300 mg. to 500 mg. of the former, depending upon its potency, and 5 drops of the latter

per day (except Sunday). Cornstarch replaced these two ingredients in the basal diet.

The animals were assigned to the test and control groups so that litter mates appeared in each group. Insofar as possible an equal distribution of sexes was made.

The depletion period averaged 5 to 6 weeks. An individual was ready for the test period when growth had definitely ceased, no gain made in 3 to 5 days, and it was considered desirable to have some evidence of ophthalmia. In practically every case an eye disturbance was observed which varied from the whitening of the edge of the lid and loss of the usual beady appearance to a complete closure of the eye with marked reddening. The ophthalmias were cured during the first week of the test period.

The material under examination was ground fine and fed as a daily supplement (except Sunday). On the basis of preliminary feeding trials it was estimated that 500 mg. of the yellow corn per rat per day and 750 mg. of the white-capped, yellow dent variety would allow an average gain of 3 gm. per week for an 8-week experimental period. For the white-capped, yellow dent variety a local strain of Mercer White-Capped, Yellow Dent was used, and a supply of yellow dent from a local dealer for the yellow variety.

#### DISCUSSION

The average net gains for the eight-week test period were 34 gm. (P.E.  $\pm$  2.9) for the group of 10 animals fed the white-capped yellow dent variety, and 40 gm. (P.E.  $\pm$  1.5) for the group of 11 fed the yellow variety. The difference between the two means, 6, is not quite twice the probable error, 3.2, of the difference between the two means (19). Hence the difference is not significant and the growth response is considered practically identical for the samples. Table I displays a summary of the results.

Since the same growth response was obtained with 500 mg. of the yellow variety as with 750 mg. of the white-capped, and both responses were less than normal, the yellow variety may be said to be about 50 per cent more potent with reference to vitamin A than the white-capped.

Although this information is available only in the case of a laboratory test animal, the white rat, it is suggested that when corn is relied upon as a source of vitamin A in a livestock ration, more of the white-capped variety be used than of the yellow, especially if the amount of the latter recommended is not much more than enough to meet the vitamin A requirements. The meal obtained by grinding white-capped yellow dent corn is of a lighter yellow color than that of the yellow dent. These findings are

in accord with those of Steenbock and Boutwell (10). The pigmentation of the white-capped variety is less than that of the yellow but greater than that of the non-pigmented, white variety, the last named variety being very deficient in the A factor according to Steenbock and Boutwell. The vitamin

TABLE I  
SUMMARY OF RAT GROWTH RECORDS, VITAMIN A TEST

Supplement	No. of animals	Av. Weight beginning of test period gm.	Av. Weight end of test period gm.	Av. Gain eight wk. test period gm.	Difference and the probable error
500 mg. yellow dent corn	11	93	133	40 (P.E.* $\pm 1.5$ )	6 $\pm 3.2$
750 mg. white-capped yellow dent	10	94	128	34 (P.E. $\pm 2.9$ )	
Controls, no supplement	13	95	Av. wt. at death—77 gm. Av. survival period—19 days		

$$* \text{P.E.} = 0.6745 \sqrt{\frac{\sum d^2}{n(n-1)}}$$

A content of the white-capped variety is intermediate between that of the yellow and white varieties.

#### CONCLUSIONS

1. Yellow dent corn was found to be about 50 per cent more potent with reference to vitamin A than a white-capped yellow dent variety.
2. The more highly pigmented variety contains the greater amount of vitamin A.
3. When the white-capped variety is used in compounding a ration, consideration should be given to the lower vitamin A content, as compared with the yellow dent variety.

#### BIBLIOGRAPHY

1. Steenbock, H., *Science*, 1919, Vol. 50, 352.
2. Drummond, J. C., *Biochem. Jour.*, 1919, XIII, 81.
3. Rosenheim, O., and Drummond, J. C., *Lancet*, 1920, I, 862.
4. Stephenson, M., *Biochem. Jour.*, 1920, XIV, 715.

5. Widmark, F., *Skand. Arch. Physiol.*, 1924, LXV, 7.
6. Palmer, L. S., *Science*, 1919, Vol. 50, 501.
7. Palmer, L. S., *Jour. Biol. Chem.*, 1915, XXIII, 261; 1916, XXVII, 27.
8. Palmer, L. S., and Kempster, H. L., *Jour. Biol. Chem.*, 1919, XXXIX, 299.
9. Drummond, J. C., and Coward, K. H., *Biochem. Jour.*, 1920, XIV, 668.
10. Steenbock, H., and Boutwell, P. W., *Jour. Biol. Chem.*, 1920, XLI, 81.
11. Steenbock, H., and Gross, E. G., *Jour. Biol. Chem.*, 1920, XLI, 149.
12. Steenbock, H., Sell, M. T., and Buell, M. V., *Jour. Biol. Chem.*, 1921, XLVII, 89.
13. Steenbock, H., Sell, M. T., and Boutwell, P. W., *Jour. Biol. Chem.*, 1921, XLVII, 303.
14. Steenbock, H., and Sell, M. T., *Jour. Biol. Chem.*, 1922, LI, 63.
15. Palmer, L. S., Kennedy, C., and Kempster, H. L., *Jour. Biol. Chem.*, 1921, LXVI, 559.
16. Sherman, H. C., and Munsell, H. E., *Jour. Amer. Chem. Soc.*, 1925, XLVII, 1639.
17. Sherman, H. C., and Campbell, H. L., *Jour. Biol. Chem.*, 1924, LX, 5.
18. Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 1919, XXXVII, 572.
19. Sherman, H. C., *Chemistry of Food and Nutrition*, New York, 1926, 3rd Ed., p. 604.
20. v. Euler, B., v. Euler, H., and Hellström, H., *Biochem. Zeit.*, 1928, CCIII, 370.
21. Duliere, W. L., Morton, R. A., and Drummond, J. C., *Chem. Ind.*, 1929, XLVIII, 518.
22. Collison, D. L., Hume, E. M., Smedley-MacLean, I., and Smith, H. H., *Chem. Ind.*, 1929, XLVIII, 631.





# THE NEPHROPATHOGENIC ACTION OF CYSTINE

## II. THE DIETARY CONTROL OF CYSTINE NEPHROSIS

BY GERALD J. COX AND LEONA HUDSON

(From the Laboratory of Physiological Chemistry,  
University of Illinois, Urbana)

Received for Publication—July 25, 1929

### INTRODUCTION

IN A recent contribution from this laboratory Cox, Smythe, and Fishback (1) have shown that "young rats of 60 gm. or less develop acute toxic nephrosis when restricted to synthetic diets containing 0.3 to 0.9 per cent of free cystine." The observations made on loss of appetite and weight, enlarged kidneys, death of some rats, and recovery of others without change of ration, were similar to those of Hartwell (2) using rations containing 20 per cent of edestin. Hartwell suggested that some specific amino acid was responsible for the indisposition of the rats and our work indicates that cystine may be the causative agent. She found that if the vitamin B of the diet, supplied by marmite, were increased sufficiently the symptoms of the disease did not appear.

Hartwell's success in preventing kidney injury due to edestin suggested the experiments reported in this communication in which we have studied the effects of a varying supply of yeast extract on the development of cystine nephrosis.

We have used the Diet C of Cox, Smythe, and Fishback as our basal ration. (See Table I.) To produce nephrosis, we have used Diet 3 in which 0.3 per cent of cystine replaces an equal amount of the casein

TABLE I  
COMPOSITION OF DIETS

	Diet C. per cent	Diet 3. per cent
Casein.....	15.0	14.7
Cystine.....	0.0	0.3
Dextrin.....	40.0	40.0
Sucrose.....	15.0	15.0
Lard.....	19.0	19.0
Cod liver oil.....	5.0	5.0
Salt mixture*.....	4.0	4.0
Agar agar.....	2.0	2.0

\* Osborne and Mendel (3).



of Diet C. It is worthy of note that the casein that has been used throughout these studies of cystine nephrosis has been a commercial casein labeled "Free from Water-Soluble Vitamin-B" supplied by the Harris laboratories. For yeast extract we have used the Osborne and Wakeman (4) vitamin-B fraction of yeast (Yeast Vitamin, Harris), supplied in two different ways. It has been fed separately in the form of pills made with 50mg. of the yeast concentrate and 100 mg. of dextrin. In other experiments it has replaced differing amounts of dextrin in the ration.

We have encountered variable resistance of rats to the effects of cystine and have attempted to determine whether it is inherent, or due to dietary influences previous to the experimental period. One group of rats from a commercial source did not show symptoms of nephrosis within ten days. Another group, obtained from another laboratory, had been born and reared on a ration containing 20 per cent of dried brewer's yeast. The rats of this latter group showed symptoms of a distinctly milder order than the rats from our colony, bred and raised on a normal stock ration.

Our procedure has been as follows. When the rats of a litter have attained body weights of about 35 gm. they are placed in individual cages and offered the experimental diet, *ad libitum*. They are weighed daily but the food intake is determined only for the total five day period. After the fifth day both food and rats are weighed daily. At the end of ten days they are killed and the kidneys removed and weighed. These kidney weights are compared with those of Donaldson (5) and those of the other rats of the litter.

In some of our first experiments the above observations were considered sufficient. But in the case of the rats in which it was desired to study the intensity of the disease, the non-protein nitrogen of the blood at death was determined by the procedure previously used (1). All kidneys were preserved in 10 per cent formaldehyde and many of them were prepared for histological examination.

### EXPERIMENTAL

As all the data of this investigation are interrelated we have condensed them to average values which we present in Table II. The response of both sexes to the diets was apparently the same. Rats from one litter were distributed among the related groups. In the columns under the heading of "Kidney Weights" we have considered the weights of Donaldson as 100 per cent. Our figures are based upon the maximum weight attained by the rat.

Our first experiment tested whether increasing the number of pills

TABLE II  
EFFECTS ON GROWTH, FOOD CONSUMPTION, KIDNEY WEIGHTS, AND NON-PROTEIN NITROGEN OF THE BLOOD OF  
A VARYING SUPPLY OF YEAST EXTRACT IN THE DIET OF YOUNG RATS CONSUMING FREE CYSTINE

Group	Yeast Extract Daily mg.	Diet	Number of rats	Average Body Weight			Food Consumed		Kidney Weights per cent of normal		Average Final blood Non-Protein N mg. per 100 cc.
				Initial gm.	Maximum gm.	Final gm.	First five days gm.	Second five days gm.	Variation	Average	
I	50	C	5	37.4	56.8	56.8	23.0	26.4	92-110	100	
II	0	3	4	36.8	52.0	50.6	23.3	16.6	167-246	204	
III	50	3	11	36.2	54.0	49.8	23.8	17.2	98-240	178	
IV	100	3	4	36.8	59.8	57.0	27.2	19.7	95-204	157	
V	150	3	4	37.0	65.0	62.8	27.8	24.5	104-221	166	
VI	300	3	4	37.8	73.3	73.3	24.4	33.1	104-117	111	
VII	0	1-V	8	37.1	53.8	48.8	24.1	16.0	107-220	177	165
VIII	0	5-V	6	36.3	68.5	68.5	25.4	32.6	87-103	93	36
IX	0	10-V	5	35.2	75.0	75.0	26.4	35.8	94-107	101	39
X	50	C	4	41.5	63.0	63.0	25.1	29.2	95-100	98	55
XI	50	3	6	36.5	56.0	55.9	21.6	19.1	105-156	125	74
XII	150	3	4	32.0	62.2	62.2	20.3	26.2	105-126	115	51
XIII	300	3	4	33.5	68.5	68.5	19.7	27.7	96-132	115	53
XIV	50	C	7	37.7	53.4	53.4	24.3	25.7	76-100	89	80
XV	50	3	9	36.0	52.9	51.5	22.3	19.3	87-198	135	138
XVI	50	C	8	35.9	50.0	50.0	20.6	22.2	85-108	95	53
XVII	50	3	11	36.6	50.8	49.9	21.1	18.2	93-222	158	121

containing 50 mg. of yeast vitamin (Harris) would prevent nephrosis. Group I (see Table II) was on the casein diet without added cystine. Group II, receiving no yeast extract, served as control. Other Groups, III, IV, V, and VI received daily 1, 2, 3, and 6 pills representing 50, 100, 150, and 300 mg. of the yeast extract. The latter was about the limiting amount that would be ingested by the rats and frequently one or two of the pills would not be consumed.

The rats of Group I did not grow rapidly due to lack of cystine in the ration but no symptoms of kidney disorder appeared. Rats of Groups II to V inclusive developed nephrosis as indicated by loss of weight and appetite during the second five day period and by kidney hypertrophy. The symptoms were progressively less intense as the yeast extract supply was increased. Four rats of Group III and two rats of Group IV died on the ninth day of the experiment, all showing the typically enlarged kidneys. Rats of Group VI receiving 300 mg. of the yeast extract showed no symptoms of the disease.

The rats of Groups V and VI at times did not consume the entire supply of the yeast extract pills especially during the first three or four days. To meet this exigency the powder was incorporated in Diet 3 replacing an equal amount of dextrin. These rations which contain 1, 5, and 10 per cent of the yeast extract are represented by Diets I-V, 5-V, and 10-V respectively. The rats of Group VII were not protected from the cystine, but those of Groups VIII and IX showed no symptoms. Based on the food intake of the first five days these latter rats received respectively about 250 and 500 mg. of yeast powder daily. Group I received about 50 mg. Two of the rats of Group I died on the ninth day of the experiment. Blood was not obtained from them and 165 mg. represents the non-protein nitrogen of the blood of the six survivors.

The rats of Group X to XIII inclusive were born and reared to the initial weights shown in the table on a ration containing 20 per cent of dried brewer's yeast. They were also from different stock than the rats of the preceding groups. Their evident resistance to the effects of free cystine in the diet led us to breed rats from our own stock but fed a ration containing 20 per cent of brewer's yeast. The rats of Groups XIV and XV were from three litters born and reared on the stock ration shown in Table III. The rats of Groups XVI and XVII are from three litters from the same parents as Groups XIV and XV but supplied throughout the breeding, gestation, and lactation periods with the yeast ration of Table IV.

Cystine nephrosis developed as usual in the rats of Groups XV and XVII ingesting free cystine, indicating that the diet previous to the ex-

perimental period has little or no influence on the susceptibility of the rats to the disease. Resistance may be hereditary but more data than we present are necessary for proof.

Examination of the kidneys of representative rats of the various groups revealed lesions, in general, the same as those reported by Cox, Smythe, and Fishback (1).

TABLE III  
STOCK RATION

	per cent
Alfalfa . . . . .	10
Meat scraps . . . . .	12
Casein . . . . .	12
Sodium chloride . . . . .	1
"Red Dog" Flour . . . . .	65

To the above diet 20 gm. of dried brewer's yeast, 20 gm. of cod liver oil, and 1 cc. of a 0.1 per cent solution of potassium iodide per kilogram were added. Milk was fed *ad libitum*. Each rat received 2 gm. of compressed baker's yeast daily, and 1 dog biscuit and 40 gm. of lettuce twice a week.

TABLE IV  
YEAST RATION

	per cent
Casein . . . . .	30
Yeast . . . . .	20
Dextrin . . . . .	46
Salt mixture* . . . . .	4
Wheat germ oil, 5 drops daily	
Cod liver oil, 5 drops daily	

\* Osborne and Mendel (3).

## DISCUSSION

It is apparent from the data presented in Table II that there is some substance present in the Osborne and Wakeman vitamin B concentrate of yeast that protects young rats from cystine nephrosis. The active factor may play a very special role in the metabolism of cystine or the excretion of that amino acid or its metabolic products. On the other hand, it may be necessary for the disposal of amino acids in general.

Hartwell's (2) experience in preventing kidney injury due to edestin by the use of autoclaved marmite indicates that the active substance is not identical with the antineuritic factor. The very special manifestation of the deficiency, the rapid development of the symptoms, the brief period of the rat's life during which susceptibility occurs, and the recovery of rats without any change in diet indicate that the active factor is not identical with any of the known accessory food substances.

Difference of opinion exists relative to the development of renal injuries due to high protein diets. As Mitchell (6) has pointed out recently, those investigators who have had positive nephritic findings have generally employed diets that were not demonstrably complete. Maclean, Smith, and Urquhart (7) have shown that rabbits on diets from which green food is excluded become nephritic and that the inclusion of cabbage leaves in the diet, which may be very high in protein, prevents kidney injury or cures it if it has developed. They have suggested that "fresh vegetables may be more important than the usual abstention from protein food" in the treatment of nephritis. Our observations on the efficacy of yeast extracts in nullifying the nephrotoxicity of cystine suggest the possible use of such active extracts in the treatment of human nephritis.

The large amount of yeast extract that we have found necessary as a preventative dose indicates either that yeast is not particularly rich in the active factor, or that the Osborne and Wakeman procedure does not concentrate it. Definite proof of the individuality of the factor which prevents the cystine nephrosis must await the exclusion of other known dietary factors, especially the anti-pellagic.

#### SUMMARY.

Cystine nephrosis in very young rats may be prevented by the inclusion in the diet of sufficient of the Osborne and Wakeman vitamin B concentrate of yeast.

The active substance of yeast extract which protects rats from cystine nephrosis is probably distinct from any of the known accessory food factors.

The susceptibility of young rats to cystine nephrosis is probably hereditary.

#### BIBLIOGRAPHY

1. Cox, G. J., Smythe, C. V., and Fishback, C. F., *Jour. Biol. Chem.*, 1929, LXXXII, 95.
2. Hartwell, G. A., *Biochem. Jour.*, 1928, XXII, 1212.
3. Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 1919, XXXVII, 572.
4. Osborne, T. B., and Wakeman, A. J., *Jour. Biol. Chem.*, 1919, XL, 383.
5. Donaldson, H. H., *The Rat*, Philadelphia, 2nd edition (1924).
6. Mitchell, H. H., *Jour. Nutr.*, 1929, I, 271.
7. Maclean, H., Smith, J. F., and Urquhart, A. L., *Brit. Jour. Exper. Path.*, 1926, VII, 360.

---

## A STUDY OF THE ANEMIA OF YOUNG PIGS AND ITS PREVENTION\*

BY E. B. HART, C. A. ELVEHJEM AND H. STEENBOCK

WITH THE CO-OPERATION OF A. R. KEMMERER

*(From the Department of Agricultural Chemistry, University of Wisconsin,  
Madison)*

and

G. BOHSTEDT AND J. M. FARGO

*(From the Department of Animal Husbandry, University of Wisconsin,  
Madison)*

Received for Publication—July 29, 1929

EVERYONE connected with the swine industry recognizes that there is a tremendous loss in pig raising due to the large number of deaths which so often occur during the early growing period. Data show that about 35 per cent of the pigs farrowed are lost during the nursing period. There are many factors which probably influence this excessive death rate, but undoubtedly one factor is the large prevalence of anemia in suckling pigs. The recognition of anemia in suckling pigs is recent and the detailed facts regarding its cause and prevention are rather meager.

McGowan and Crichton (1, 2) were pioneer investigators in this field. They not only observed the presence of anemia in suckling pigs when confined indoors, but also studied the symptoms of this disease and made suggestions as to its prevention. These workers noted that anemia was exceedingly prevalent in British pig breeding establishments where the sows were brought into houses, put in pens with concrete floors, and fed a dry ration during the farrowing period. The sows would farrow normally but when the pigs were from three to four weeks old, they took on a "stocky" appearance due to oedema of the skin. The breathing became pumping in character and "thumps," a spasmodic jerking of the diaphragm, developed. Death was common and often entire litters perished. On post-mortem examination, the heart was found to be greatly dilated, there was an excess of pericardial fluid, and the lungs were oedematous with effusion into the pleural cavities. The blood was extremely watery and pale, the hemoglobin often being as low as 15 per cent of the normal. In the cases which continued to live, the animals became very emaciated, ceased to grow, and

---

\* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

lost their appetites. Diarrhoea was present in a large number of the pigs.

McGowan and Crichton believed the chief cause of this disease to be an iron deficiency. When the sows were out of doors they obtained abundant iron from the grass and the soil, but when they were kept indoors, owing to the concrete floors and the nature of the food, the supply of iron decreased. Excellent results were obtained both in the amelioration of the anemia from which the pigs were already suffering and in its prevention in the younger pigs when large doses of ferric oxide were fed to the sow. The British workers were in doubt as to whether the beneficial effect noted in the young pigs when the sows were fed ferric oxide was due to a more abundant source of iron in the refuse and feces of the sow, or to an increased iron content of the sow's milk. They suggest that the effect is probably not due to any change in the composition of the milk.

This specific question was one of the reasons which prompted us to investigate the effect of diet on the iron content of milk. We were unable to make this investigation on sow's milk but we did find that increasing the iron content of the rations fed to cows and to goats did not increase the iron content of the respective milks (3). Undoubtedly this fundamental fact also can be applied to the pig.

Doyle, Mathews, and Whiting (4) have observed the same condition as reported by McGowan and Crichton in young suckling pigs reared by sows kept indoors. These workers have made an excellent study of the symptoms of this disease, including both the gross and microscopic pathological changes. They concluded that the anemia was not influenced by the vitamin supply in the ration nor by the addition of ferric lactate, but it was markedly affected by some factor in outside conditions. Although they did not analyze the out-of-door factor which played an important part in hemoglobin formation, they have demonstrated by recent work (5) that irradiation by means of a quartz mercury vapor lamp has no beneficial effect on the hemoglobin content of the blood of young pigs.

An extensive study of the factors affecting hemoglobin synthesis in several species of animals when restricted to milk diets has been underway at this station during the past five years. Definite knowledge of these factors was gained when it was demonstrated that copper is essential as a supplement to iron for normal hemoglobin synthesis (6).

With this fundamental fact in mind we wished to extend our studies to the pig in addition to the rabbit, the chick, and the rat. We have demonstrated that the rabbit and the rat must be restricted to a milk diet for some time after the normal weaning period in order to produce a severe anemia. We were interested, therefore, in determining if the pig differs from these

animals and is susceptible to severe anemia during the suckling state. If this is true, then a study of the factors which will prevent this condition is of much value. The effect on the hemoglobin content of the blood of suckling pigs, of feeding iron and copper salts to the sow during the gestation period, and to the young after their birth, is an important question to be answered.

## EXPERIMENTAL

### *Work with Pigs after Weaning Period*

The first part of this study consisted of following the hemoglobin content of the blood of pigs placed, shortly after weaning, on a diet quite low in iron. Iron and copper additions were also made to this ration comparatively low in iron, to determine if the hemoglobin content could be modified by these salts. Thirty gilts weighing from 40 to 60 pounds were selected from the general herd of pigs at the University swine barn. They were started on the experiment June 19, 1928, which was about two weeks after most of the pigs had been weaned. The thirty gilts were divided into ten lots of 3 pigs each in such a manner that each lot contained one Poland China, one Duroc Jersey, and one Chester White pig. All the groups were kept indoors and each lot placed in a pen with concrete floor and partial wooden overlay. The pens were equipped with a concrete trough for feeding purposes.

The basal ration consisted of yellow corn 99 parts, common salt 1 part, and skim milk; 3 pounds of the latter were fed for each pound of grain mixture. Each pig was also allowed 50 cc. of cod liver oil per week. One lot received only the basal ration while varying amounts of iron and copper additions were fed to the other lots. The additions made to the rations fed each of the groups were as follows:

Group 1, Basal

Group 2, Basal plus 10 mg. iron per pig daily

Group 3, Basal plus 50 mg. iron per pig daily

Group 4, Basal plus 250 mg. iron per pig daily

Group 5, Basal plus 2 mg. copper per pig daily

Group 6, Basal plus 50 mg. iron and 2 mg. copper per pig daily

Group 7, Basal plus 50 mg. iron and 10 mg. copper per pig daily

Group 8, Basal plus 50 mg. iron and 20 mg. copper per pig

Group 9, Basal plus 50 mg. iron and 50 mg. copper per pig daily

Group 10, Basal plus  $\text{Fe}_2\text{O}_3$ , 0.5 pound per 100 pounds grain mixture and 2 mg. copper per pig daily.



All the iron additions except in Lot 10 were made as ferric sulphate,  $\text{Fe}_2(\text{SO}_4)_3$ , and all the copper additions were made as copper sulphate  $\text{CuSO}_4$ . The salts were dissolved in water and made to definite volumes. Amounts of these solutions equivalent to the quantity of iron and copper to be fed were added to the milk daily.

Weights of the animals were taken weekly. Hemoglobin determinations were made at the beginning of the experiment and every two weeks thereafter during the first part of the experiment, and every month during the latter part of the study. The blood was obtained by tail bleeding and the hemoglobin determined by the Newcomer method.

The results of the hemoglobin determinations are given in Table I. Each figure is the average of the hemoglobin values for the three sows in one lot.

A study of the figures shows that the great majority of them fall between 8-10 grams per 100 cc. of blood. This range is undoubtedly the normal hemoglobin value for pigs. The hemoglobin values for the three sows receiving the basal ration alone are well within this range. This ration, although low in iron, must furnish a sufficient amount, together with what may be picked up in the refuse, to allow normal hemoglobin building during the period of growth following weaning. If these pigs had been anemic during the suckling stage, they had improved enough by the time the experiment was started to show a normal blood stream. The hemoglobin content of the blood from sows receiving varying additions of iron and copper was no higher than that of the sows receiving the basal ration. The sows fed the  $\text{Fe}_2\text{O}_3$  gave hemoglobin values slightly lower than the other sows. We have no explanation for this result. Another interesting fact is that the hemoglobin values for all groups were the lowest during the second month on the ration, which is probably due to the rapid rate of growth during this time.

We used all the sows in this experiment because then we could study the effect of these additions upon the hemoglobin values in the young produced by the sows on the various rations. The sows were therefore bred during the winter so that the young would be farrowed during April and May.

#### *Work with Pigs During the Suckling Period*

The first sow to farrow was No. 520 in Group 1. We present the curves for the hemoglobin values of two of the eleven pigs in this litter in Chart 1. These curves are typical of the blood changes which took place in all the pigs of this litter. Every pig in this litter which received no addition to the mother's milk was dead within seven weeks.

TABLE I  
THE EFFECT OF IRON AND COPPER ADDITIONS TO A BASAL RATION FED TO GROWING SOWS.  
Sows were started on the different rations shortly after weaning.  
\*Grams Hemoglobin per 100 cc. Blood

No. of ration	Days on Ration									
	0	14	28	40	74	105	138	167	216	Average
1	8.78	9.07	7.53	7.33	7.76	8.58	8.47	11.05	9.80	8.7
2	7.98	9.08	7.65	7.58	7.78	7.92	9.53	8.41	10.23	8.7
3	8.67	9.45	7.70	7.34	7.05	8.44	7.72	9.81	10.77	8.8
4	8.81	9.21	8.17	7.79	7.58	7.39	8.18	10.08	9.57	8.4
5	8.23	8.96	8.42	8.38	9.42	10.39	11.14	9.36	10.36	9.4
6	8.76	9.18	7.81	8.72	8.48	9.92	10.74	11.06	10.03	9.4
7	8.44	8.87	6.78	6.93	7.62	9.20	11.11	10.93	9.40	8.8
8	8.73	8.78	6.76	7.06	6.96	8.58	10.13	10.12	10.36	8.7
9	8.55	8.15	7.69	8.51	8.96	9.26	10.27	11.90	10.94	9.4
10	7.95	6.30	6.69	5.78	7.29	7.29	8.02	8.56	9.08	7.4

\* The hemoglobin figures are averages of the values for each of the three sows on the individual rations.

We were not surprised to find this picture with the pigs farrowed from this sow because she had received only corn and milk which are low in iron and the low intake of this element may have caused low iron reserves in the young. However, we were surprised to find that every litter farrowed by these sows, without regard to the iron and copper addition to the basal ration, exhibited identical blood changes. Chart 1 gives a curve drawn from the average of the hemoglobin values of all the pigs which did not receive any additions after birth. The blood changes are identical with those described by McGowan and Crichton and by Doyle and coworkers. The other symptoms observed were also so similar to those described by these workers that it is unnecessary to discuss them further.

It is very important to note that pigs farrowed from sows receiving large additions of iron and copper develop anemia just as rapidly as the pigs farrowed from the sows on the basal ration. For the purpose of comparison we also present in Chart 1 the curves of the hemoglobin changes in two pigs from the litter farrowed by Sow No. 331 in Group 9, which had received daily additions of 50 mg. of iron and 50 mg. of copper during the entire gestation period. The slope of these curves is just as great as that of the upper curves shown in Chart 1 representing the pigs from the sow on the basal ration.

The facts demonstrate that the rate of anemia development in young suckling pigs is not affected by liberal additions of iron and copper to the ration of the sow. We can conclude therefore that the reserve supply of iron and copper in the pig at birth cannot be increased by feeding the sow these salts. The analysis of the livers taken from some of the pigs at birth gives results which agree with this conclusion. The livers were removed from the pigs directly after birth, dried, and analyzed for iron and copper. A few of the results for pigs farrowed by sows fed the basal ration and sows fed this ration plus iron and copper additions are given in Table II. The iron content of the livers from the pigs farrowed by sows receiving 50 mg. iron daily was no higher than the iron content of the livers from the pigs farrowed by sows on the basal ration. The average of the figures for the copper content of the livers from sows receiving high levels of this element is slightly higher than the figures for the livers from the pigs farrowed by sows on the basal ration. However, the figures are not strikingly different, and more data are required before one could conclude that the copper content of the livers of young pigs at birth is influenced by the copper content of the sow's ration. The inability to definitely increase the reserve of blood forming elements in the pig at birth substantiates the findings in this laboratory of Lindow, Peterson, and Steenbock

(7) who showed that feeding large amounts of copper to rats during the gestation period did not delay the development of anemia in the young and did not increase the copper content of the young at birth. We can also conclude that the iron and copper content of the sow's milk is not influenced by the ingestion of these salts. This conclusion is in accord with our recent work (3, 8) which demonstrated that the iron and copper content of cow's milk and goat's milk cannot be increased by feeding iron and copper salts in the rations of these animals.

TABLE II  
THE EFFECT OF THE SOW'S RATION ON THE IRON AND COPPER  
CONTENT OF THE LIVER OF THE PIGS AT BIRTH

Pig No.	Farrowed by sow in Group	Daily additions to the sow's ration		Iron content of dried liver per cent	Copper content of dried liver per cent
		Iron	Copper		
2	I	none	none	0.1520	0.0163
11	I	none	none	0.1160	0.0183
R. 1	V	none	2 mg.	0.0895	0.0254
R. 2	V	none	2 mg.	0.0946	0.0294
R. 3	V	none	2 mg.	0.1270	0.0200
42	VIII	50 mg.	20 mg.	0.0974	0.0254
43	VIII	50 mg.	20 mg.	0.2020	0.0305
45	VIII	50 mg.	20 mg.	0.1740	0.0320
B. 1	IX	50 mg.	50 mg.	0.1280	0.0341
B. 2	IX	50 mg.	50 mg.	0.0860	0.0306
B. 3	IX	50 mg.	50 mg.	0.0904	0.0320

We felt that a study of the effect of the ration on the rate of anemia development in the young was of such practical importance that the study should not be confined to restricted rations supplemented with iron and copper but should also include more practical rations. Therefore nine additional sows were selected from the general herd shortly after they were bred and divided into three lots of three sows each. Lot I was placed on the regular winter ration used by the Swine Department, consisting of yellow corn 35 parts, oats 29.5 parts, wheat middlings 20 parts, tankage 5 parts, oil meal 5 parts, chopped alfalfa 5 parts and salt 0.5 parts. Lot II received the basal ration of milk and corn used for the other series of sows and Lot III the same basal ration plus 100 mg. of Fe and 20 mg. of Cu per pig daily. These sows also received cod liver oil for protection against rickets.

When these sows farrowed, the hemoglobin of the young was followed

to note any difference in the rate of anemia development. The amount of hemoglobin in the pigs at birth was normal as we had found with all the other pigs. The average hemoglobin at birth for the pigs in the three lots was as follows: Lot I, 7.77 gm.; Lot II, 7.91 gm.; Lot III, 7.42 gm. As the pigs grew they all developed anemia regardless of the ration fed to the sow. The curves given in Chart 2 show that pigs farrowed from a sow fed a practical ration come down with anemia just as rapidly as the pigs from the sows fed the basal ration.

#### *Treatment of Anemia in the Suckling Stage*

Since the reinforcement of the sow's ration with iron and copper does not protect the young pig, the problem centers around the direct feeding of these elements to the suckling pig. Solutions of  $\text{Fe}_2(\text{SO}_4)_3$  were prepared so that 1 cc. contained 25 mg. of Fe and solutions of copper sulfate were made so that 1 cc. contained 5 mg. of Cu. The ferric sulfate used contained small amounts of copper as an impurity. Dosages of 1 cc. of the solutions were then fed to the pigs with the aid of a pipette. Some of the pigs were given the iron alone, others were fed both the iron and the copper. The hemoglobin curves for four pigs from Sow 406 which are litter mates of pigs 109 and 110 whose hemoglobin changes are shown in Chart 2 are given in Chart 3. Pigs 112 and 113 received 25 mg. of Fe per pig daily and pigs 114 and 115 received 25 mg. of Fe and 5 mg. of Cu daily. Chart 4 similarly shows the hemoglobin changes in four pigs from Sow 306 which are litter mates of pigs 101 and 102 whose curves were shown in Chart 2. Pigs 103 and 104 were fed iron alone and pigs 105 and 106 were given both iron and copper. When the curves presented in these charts are compared with those in Chart 1 for the pigs restricted to sow's milk only, the effectiveness of the iron and copper treatment is definitely established. The response obtained by either iron alone or both iron and copper was exceptionally rapid, the hemoglobin reaching the normal level in two weeks and remaining at that value as long as the experiments were continued.

The iron and copper feeding to the suckling pigs not only stimulated hemoglobin synthesis but also increased the amounts of these elements in the livers of the young pigs. In Table III are given the quantities of iron and copper present in the livers taken from the pigs farrowed by Sow No. 306 when the young were 51 days old. Two pigs in this litter had received only sow's milk during this period, two had received 25 mg. of iron and two had received 25 mg. of iron and 5 mg. of copper during the last 45 days of the period. The iron content of the livers from pigs 101 and 102 had decreased to about 1/10 the amount normally present in the

liver at birth. Similarly the copper content decreased to about 1/5 of the amount found in the livers at birth. The iron content of the livers from the four pigs which received the iron additions for 45 days had increased to three times the amount in the livers from anemic pigs. The copper content of the livers from the two pigs fed iron alone was not increased, but the livers from the two pigs fed both iron and copper contained about three times the amount of copper found in the livers of the anemic pigs. However, the figures for the iron and copper content did not reach the amounts found in the liver at birth. It is important to note that the hemoglobin regeneration was rapid in the pigs receiving the iron alone, even though the copper content of the livers was not increased.

TABLE III  
THE EFFECT OF IRON AND COPPER FEEDING TO SUCKLING PIGS ON  
THE QUANTITY OF THESE ELEMENTS IN THE LIVER

Pig No.	Pig's Ration	Iron in dried liver per cent	Copper in dried liver per cent
101	Milk alone	0.0128	0.00373
102	Milk alone	0.0100	0.00450
103	Milk plus iron	0.0330	0.00431
104	Milk plus iron	0.0314	0.00650
105	Milk plus iron and copper	0.0386	0.01300
106	Milk plus iron and copper	0.0296	0.00880

Further proof of the effectiveness of iron feeding is shown in Table IV. Hemoglobin values for the entire litter from Sow No. 331 are tabu-

TABLE IV  
THE EFFECT OF IRON AND COPPER FEEDING ON THE HEMOGLOBIN CONTENT OF THE BLOOD OF  
SUCKLING PIGS. RECORD OF SOW NO. 331 WHICH FARROWED 7 PIGS MAY 6, 1929  
Grams Hemoglobin per 100 cc. Blood

No. of Pig	Age in Days									
	1	7	14	15	23	30	38	46	53	60
42	7.94	4.30	2.75	—	3.57	dead				
43	8.28	5.47	3.77	—	3.48	3.55	3.15	2.96	3.53	2.71
44	5.69	3.25	2.12	Iron added	6.37	7.08	8.79	9.66	10.73	10.36
45	8.52	5.69	3.08	Iron added	6.74	8.40	9.35	8.79	11.82	9.21
46	8.65	5.37	3.38	Iron and copper added	6.45	7.62	8.40	9.84	10.94	9.66
47	8.92	4.96	2.92	Iron and copper added	6.04	7.83	8.52	9.48	11.82	10.94

lated, which show the condition of the blood stream in two pigs allowed only the sow's milk, in two pigs receiving iron additions, and in two pigs getting both iron and copper supplements. Again we see that very rapid improvement takes place in the blood stream, whether the iron is fed alone or the iron is supplemented with copper. The records presented in the table and the charts are typical of the improvement noted in all the pigs which received the iron treatment.

These results are in accord with those obtained by McGowan and Crichton (2) when they fed ferric oxide. These workers fed large quantities of ferric oxide to the sow and found that the anemia in the young was prevented by this treatment. The young pigs were undoubtedly benefited by the consumption of some of the iron in the feces. The iron in the ferric oxide must have been made more available during its passage through the digestive tract of the sow, because we have found that feeding ferric oxide directly to the young ameliorates anemia very slowly. In Table V

TABLE V

THE EFFECT OF FERRIC OXIDE FEEDING ON THE HEMOGLOBIN CONTENT OF THE BLOOD OF SUCKLING PIGS. RECORD OF SOW NO. 382 WHICH FARROWED 6 PIGS MAY 15, 1929

Grams Hemoglobin Per 100 cc. Blood

No. of Pig	Age in Days							
	1	10	19	27*	37	42	49	56
140	6.60	3.98	5.42	4.53	5.86	3.24	3.48	3.79
141	9.85	5.98	6.04	4.92	3.40	5.52	6.99	7.08
142	7.08	4.76	5.52	5.08	4.27	4.88	5.98	4.76
143	7.08	3.66	4.23	4.53	3.98	5.47	5.80	5.52
144	6.24	3.48	2.93	2.76	3.57	5.47	4.06	4.23
145	6.17	5.13	dead					

\* Iron additions equivalent to 125 mg.  $\text{Fe}_2\text{O}_3$  per pig started 27th day.

are given the figures which show the blood changes in anemic pigs when they were fed daily 125 mg. of  $\text{Fe}_2\text{O}_3$  by capsule. These results show that the best means of preventing the anemia, or curing it after such a condition exists, is the feeding of a soluble iron salt directly to the young pig.

#### *Work with Pigs Fed Cow's Milk*

In all the work with the suckling pigs care was taken that the pigs were restricted entirely to sow's milk, *i.e.*, access of the young pig to the sow's ration was prevented as much as possible. The sows were removed to other pens when they were fed, thereby eliminating the presence of

food in the pens where the young pigs were kept. Even when these precautions were taken the pigs had access to the sow's feces. In order to eliminate this difficulty and to determine if iron and copper additions would bring about hemoglobin regeneration under more restricted conditions, some of the pigs were taken from the sow when 3 weeks old and fed cow's milk. Incidentally this procedure allowed us to determine what effect cow's milk has on the rate of anemia development.

In Chart 5 we present the hemoglobin values for three pigs; one on cow's milk alone, one on cow's milk plus an iron addition, and one on cow's milk plus both iron and copper additions. The iron used for these pigs was a purified  $\text{FeCl}_3$  free from copper. We wished to determine if iron alone would prevent the anemia in the conditions under which these pigs were fed, or if it was necessary to add both iron and copper. It is seen from the chart that a pig becomes just as anemic when maintained on cow's milk as when allowed the sow's milk. The pig fed the pure  $\text{FeCl}_3$  made almost as rapid recovery as the one fed both iron and copper. There may be two reasons for this fact. First, the iron reserves in the young pig may be depleted first and as soon as this element is supplied, hemoglobin synthesis takes place without the additions of copper. Second, the pigs were not placed under as restricted conditions as were the smaller animals used in our earlier experimental work, and were probably able to acquire small amounts of copper.

From a practical point of view, the feeding of iron is the important preventative of anemia in suckling pigs. However, it is entirely possible that the importance of copper can be demonstrated by keeping the pigs under very restricted conditions and allowing them to remain anemic for some time to insure a heavy drain on the copper reserves.

These pigs were kept indoors with no access to sunlight or other outside factors; still both the pigs fed the iron and the one fed iron and copper grew normally and were normal in every way when the experiment was discontinued. This shows that iron together with copper present in the reserves of the animals or acquired from the refuse will correct all the hemoglobin-forming deficiencies in sow's milk and that outside factors are not necessary for the prevention of anemia in suckling pigs. Further proof of this fact is given by the following work. Nine pigs were taken from the sow when about three weeks old and placed on cow's milk. When all the pigs were anemic, two were left on the basal ration of cow's milk; two were fed the basal ration plus 25 mg. of Fe as pure  $\text{FeCl}_3$  daily; one was fed 25 mg. of Fe daily as pure  $\text{FeCl}_3$  and 5 mg. of Cu as  $\text{CuSO}_4$ ; two were placed on the basal ration and exposed ten minutes daily to a



quartz mercury vapor lamp; and two were kept on the basal ration and exposed thirty minutes daily to sunlight. The results of this work are given in Table VI. The response to the iron feeding is identical with all other pigs given iron, but it is readily seen that the ultraviolet light and sunlight are not effective in promoting hemoglobin synthesis.

TABLE VI  
THE EFFECT OF DIFFERENT TREATMENTS ON THE ANEMIA IN PIGS  
RESTRICTED TO COW'S MILK  
Grams Hemoglobin Per 100 cc. Blood

No. of Pig	Days After Treatment was Started				
	0	Treatment	8	15	22
432	5.42	Basal	5.37	5.32	4.17
434	4.92	Basal	5.37	5.04	6.11
435	4.43	Iron	6.31	6.67	7.62
433	5.98	Iron	8.28	8.34	10.01
430	4.76	Iron plus copper	8.28	10.01	11.54
1435	3.89	Ultra violet light	3.66	3.29	2.98
439	4.68	Ultra violet light	4.15	4.30	4.37
437	3.79	Sunlight	2.64	2.25	2.00
438	3.22	Sunlight	2.37	2.00	2.00

### DISCUSSION

The demonstration of the presence of anemia in practically 100 per cent of the pigs studied may raise the question of why still greater losses are not encountered in pig raising. It is well to remember that all the pigs used in this study were restricted in pens with practically no access to other food than the sow's milk. When pigs are farrowed out of doors they start to root in the soil and consume green grass and soil material at a very early period in life. Apparently, in most cases, enough iron and copper are consumed to prevent severe anemia. When the pigs are kept indoors they become very anemic in three weeks time, if they do not consume additional food. In some cases we have noted gradual regeneration of the blood stream when the pigs consumed only small amounts of the sow's ration. If large amounts were consumed and the ration were richer in iron, the regeneration would undoubtedly have been much more rapid. However, in many cases the pigs succumb to anemia before they are old enough to hunt their own food.

The simplest way to insure against anemia is to allow the young pig sufficient iron. Just how the iron is supplied, whether by the direct ad-

ministration of iron or by the reinforcement of easily accessible food, apparently makes little difference. The solution of the entire problem lies in supplying the pig with iron during the period when milk constitutes the sole ration.

### CONCLUSIONS

Sows restricted, shortly after weaning, to a yellow-corn-skim-milk diet and to floored pens maintain a normal hemoglobin level during the entire growing period. The addition of varying amounts of iron and copper to the ration does not increase the quantity of hemoglobin in the blood.

The pigs farrowed by these sows invariably develop a severe anemia in 3 to 4 weeks. The hemoglobin falls from a normal of 8 gm. per 100 cc. of blood at birth to 3 to 4 gm. per 100 cc. in a period of a few weeks. *The feeding of considerable amounts of iron and copper to the sow does not delay the development of anemia.*

Pigs farrowed by sows fed a practical winter ration developed anemia as rapidly as those from sows fed the basal ration of milk and corn.

The anemia in the suckling pigs is rapidly cured by the administration of iron and copper salts. The feeding of iron alone stimulates hemoglobin synthesis as well as when the iron is supplemented with copper.

Suckling pigs changed to a diet of cow's milk continue to develop anemia as rapidly as when allowed to remain with the sow. Under the experimental conditions, which we could establish with these pigs, the addition of pure  $\text{FeCl}_3$ , copper free, to the cow's milk, cures the anemia and maintains the pig in normal condition. The exposure of pigs on a cow's milk diet to ultraviolet light and sunlight does not stimulate hemoglobin synthesis.

The severe anemia prevalent in suckling pigs kept indoors can readily be cured or prevented by administration of soluble iron salts.

### BIBLIOGRAPHY

1. McGowan, J. P., and Crichton, A., *Biochem. Jour.*, 1923, XVII, 204.
2. McGowan, J. P., and Crichton, A., *Biochem. Jour.*, 1924, XVIII, 265.
3. Elvehjem, C. A., Herrin, R. C., Hart, E. B., *Jour. Biol. Chem.*, 1927, LXXI, 255.
4. Doyle, L. P., Mathews, F. P., Whiting, R. A., *Jour. Amer. Vet. Medical Assoc.*, 1927-28, LXXII, 491.
5. Mathews, F. P., Doyle, L. P., Whiting, R. A., *Amer. Jour. Physiol.*, 1929, LXXXVIII, 616.
6. Hart, E. B., Steenbock, H., Waddell, J., Elvehjem, C. A., *Jour. Biol. Chem.*, 1928, LXXVII, 797.
7. Lindow, C. W., Peterson, W. H., Steenbock, H., *Jour. Biol. Chem.*, 1929, LXXXIV, 419.
8. Elvehjem, C. A., Steenbock, H., Hart, E. B., *Jour. Biol. Chem.*, 1929, LXXXII, 27.

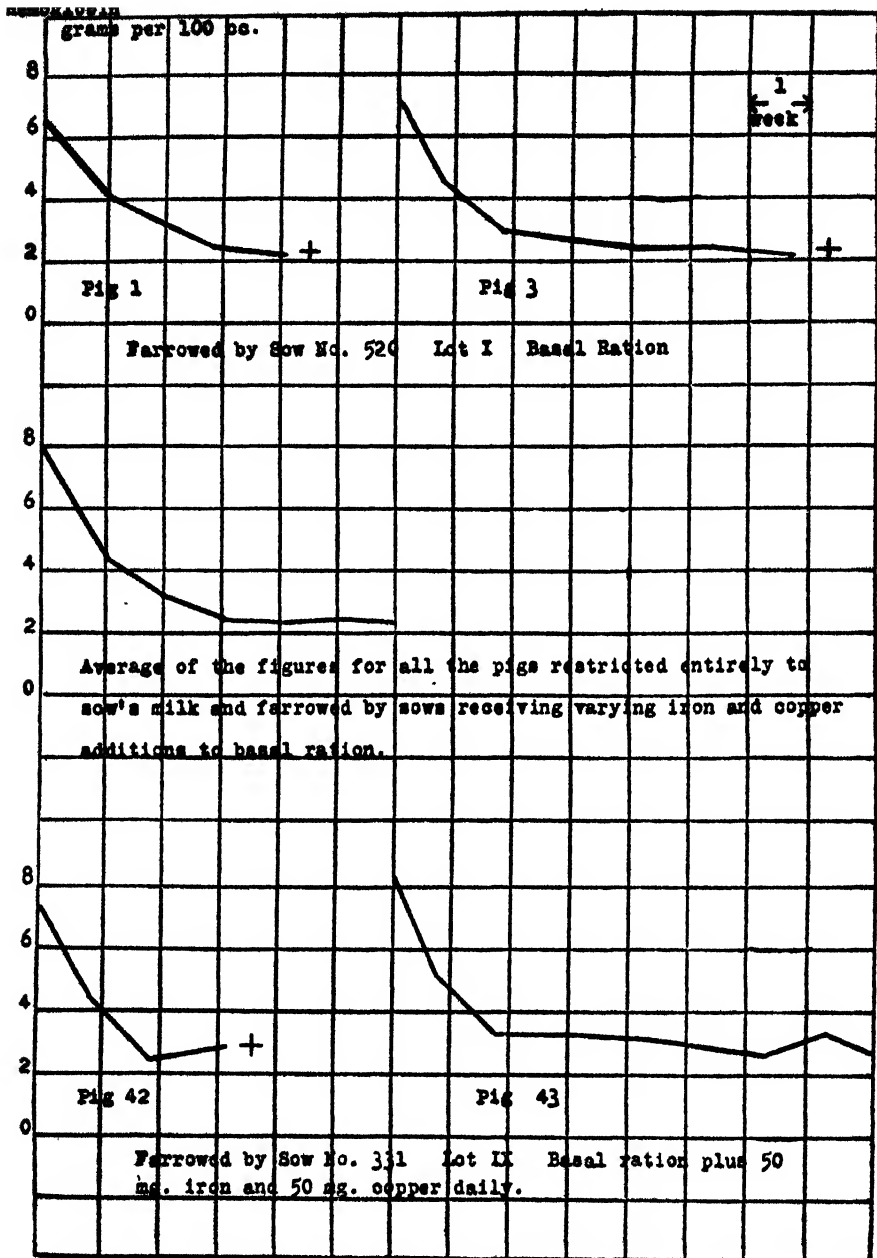


CHART 1.—In this chart are presented the hemoglobin curves for pigs restricted entirely to sow's milk and farrowed by sows receiving different iron and copper additions to a basal ration of yellow corn and skim milk. Hemoglobin determinations were made at weekly periods. The rate of anemia development in suckling pigs is not affected by the iron and copper intake of the sow. The cross at the end of the curve denotes death.

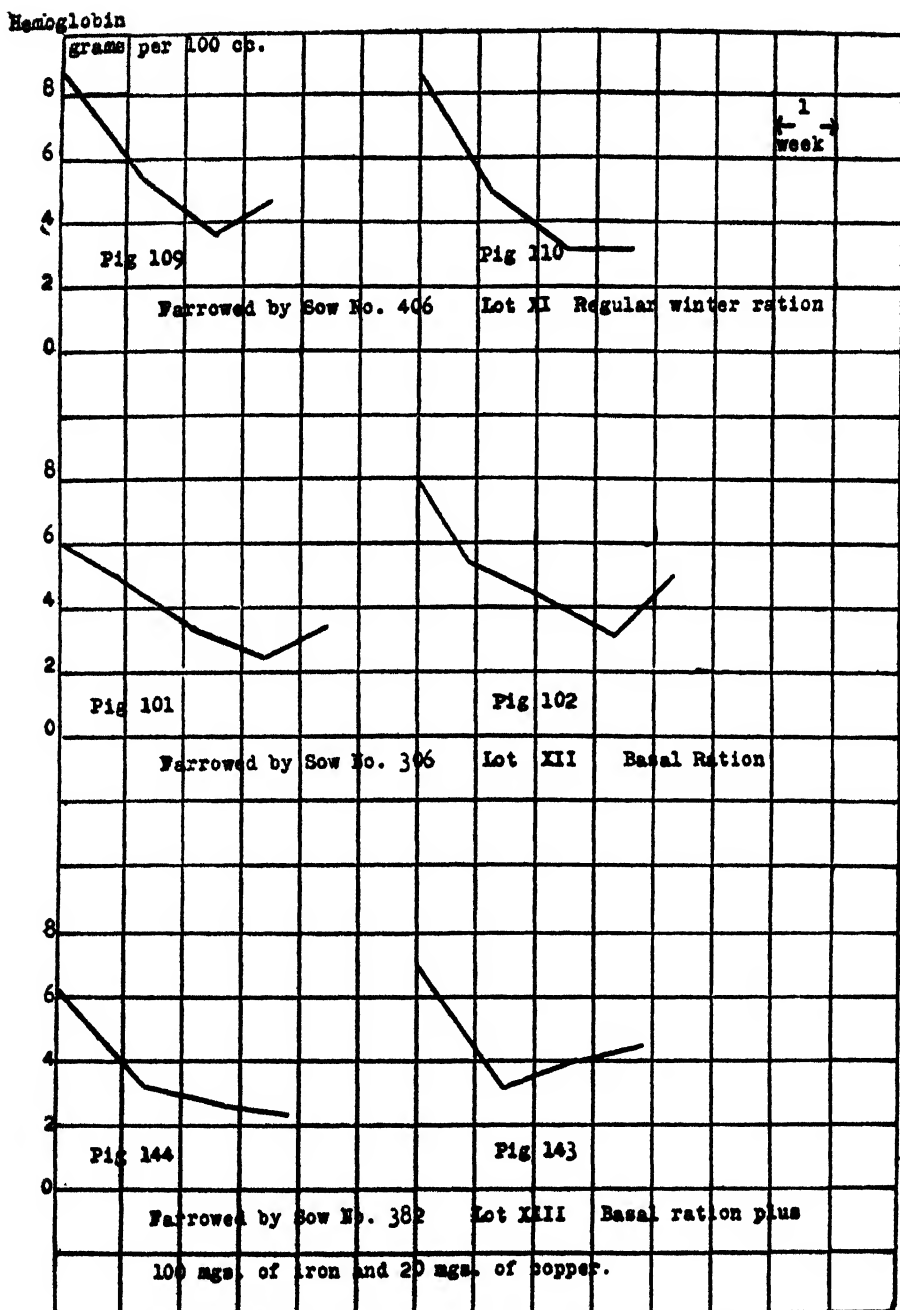


CHART 2.—The curves in this chart show the decrease in the hemoglobin content of the blood of pigs farrowed by sows on different rations when restricted entirely to sow's milk. The diet of the sow has no effect on the rate at which anemia develops in the young pigs.

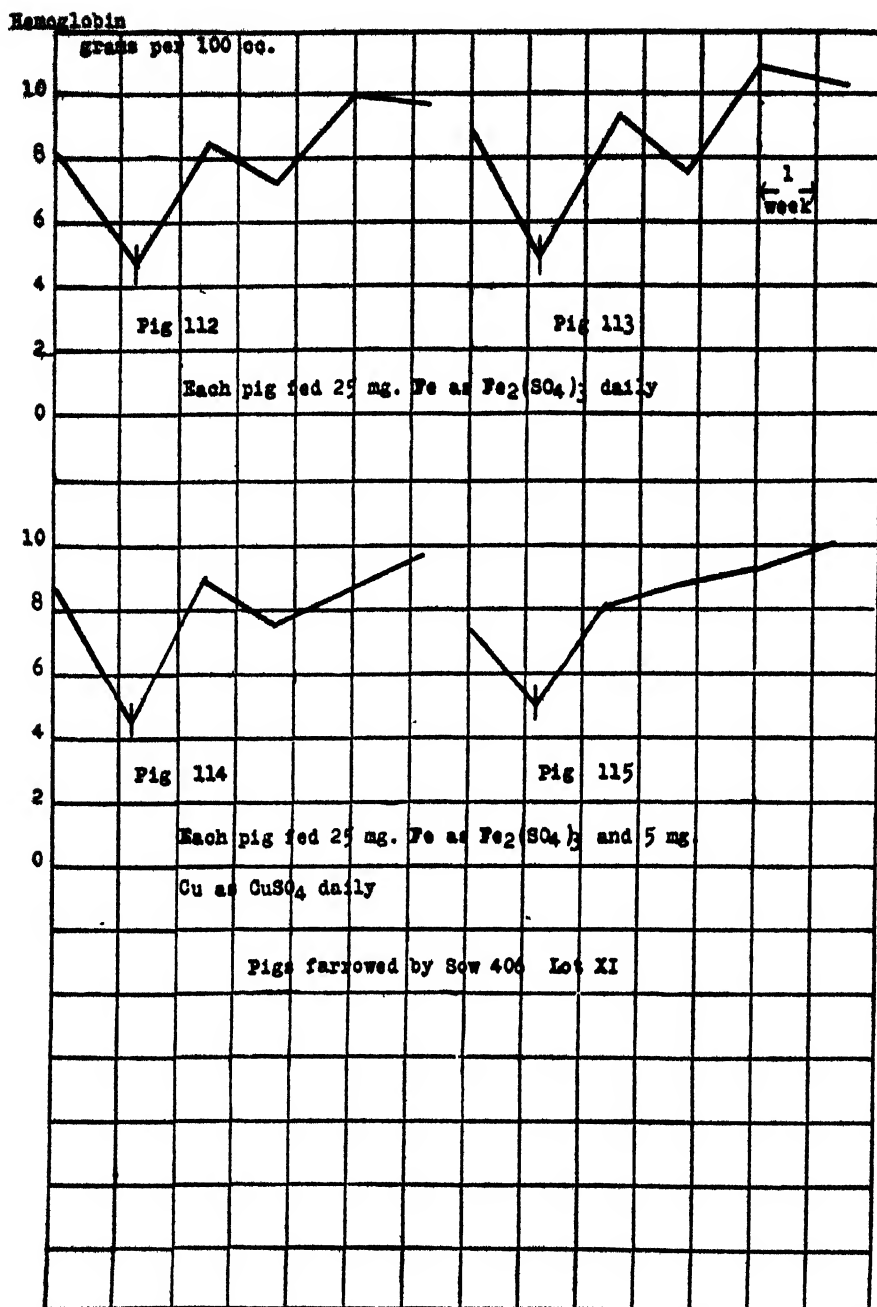


CHART 3.—The above curves show the rapid improvement in the hemoglobin of the blood of pigs when iron alone or iron plus copper is fed to the young pigs restricted entirely to sow's milk. The line across the hemoglobin curve indicates the point at which the additions to the diet were made.

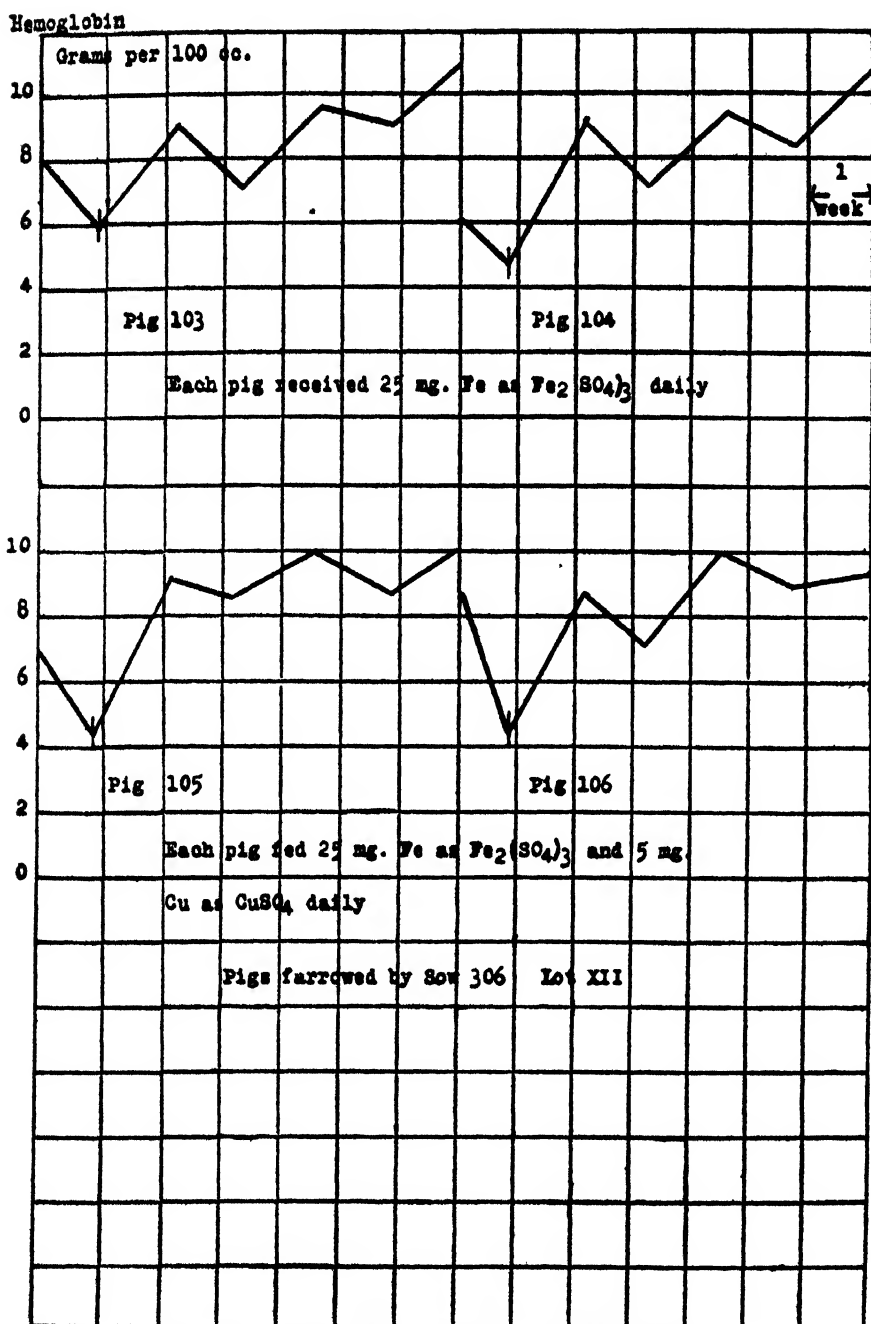


CHART 4.—Further curves are given in this chart showing the effect of iron and copper administrations to young pigs restricted entirely to sow's milk. The line across the hemoglobin curve indicates the point at which the additions to the ration were made.

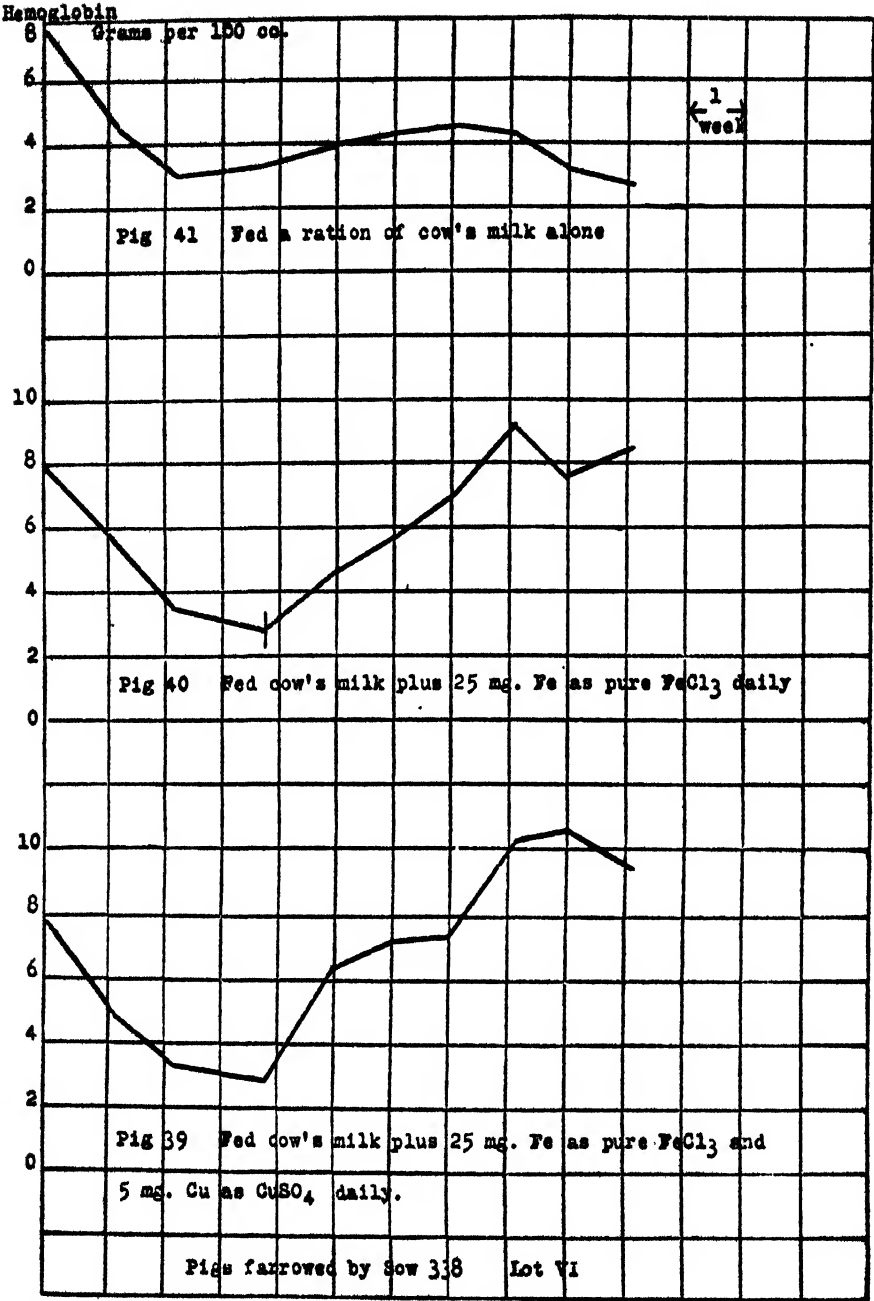


CHART 5.—The above curves show the effect of iron and copper additions to pigs restricted to a ration of cow's milk. The hemoglobin of the blood is maintained at a normal level whether pure  $\text{FeCl}_3$  is fed alone or whether it is supplemented with copper. The line across the hemoglobin curve indicates the point at which the additions to the ration were made.



## THE CAUSE OF THE LAXATIVE ACTION OF BRAN

BY MARK FALCON-LESSES

(From the Department of Internal Medicine, Medical School, University of Michigan, Ann Arbor.)

Received for Publication—August 6, 1929

WHILE the laxative nature of bran has been appreciated since the days of Graham (1794–1851), the causation of its laxative action has received desultory study since the first investigation of Jordan, Patten, and Hart (1) in 1906. Chemical analysis of bran shows that it contains many substances which might act as laxatives. Some of these constituents are: 1.—Undigested carbohydrates such as crude fiber and pentosans occurring as part of the cell membrane. 2.—The ash of bran, which amounts to about 6 per cent of the unwashed bran and is distributed in part as follows according to Sherman: (2)

Calcium...	0.120	per cent
Magnesium	0.511	" "
Potassium...	1.217	" "
Sodium.....	0.154	" "
Phosphorous	1.215	" "
Chloride	0.090	" "
Sulphur..	0.247	" "
Iron.....	0.0078	" "

It can readily be seen that there is an abundance of minerals definitely known to be laxative (as *e.g.* magnesium and sulphur). 3.—A compound of phosphorous and carbohydrate known as phytin to which has also been ascribed potency as a laxative.

### SUMMARY OF PREVIOUS REPORTS

One of the earliest experimental reports dealing with the laxative nature of bran was by Jordan, Patten and Hart in 1906 (1). They were the first to suggest that bran owed its laxative nature to a contained phosphorous compound known as phytin. Certainly this seemed to be the case with cows for, they write: "In our attempts at transition from one ration to the other we soon learned that it was not possible to change the cow suddenly from a ration rich in phytin to one low in this compound and keep the animal in normal health. Such a change caused her to become seriously constipated and recourse to a purgative was necessary." They secured a ration rich in phytin by using unwashed bran and one low or absent by using wheat bran which had been allowed to soak over night



in water slightly acid to litmus. They further studied the metabolism of the phosphorous contained in phytin and concluded that after being reduced to inorganic phosphorous combinations it was excreted chiefly in the feces and slightly in the urine.

Their analyses of both the unwashed and washed bran showed that the washing had removed the greater portion of the magnesium and potassium compounds and but very little of the calcium compounds. They give this table:

INORGANIC CONSTITUENTS OF BRAN BEFORE AND AFTER LEACHING

	P %	CaO %	MgO %	K <sub>2</sub> O %	Totals %
Whole bran . . . . .	1.420	0.182	0.894	1.580	4.076
Washed bran . . . . .	0.145	0.380	0.162	0.084	0.971

It is thus seen that bran washed in acidified water not only contains little phytin but also has a markedly lower mineral content, especially of such laxative mineral compounds as the magnesium salts.

They suggested that the true source of the laxative action of bran lay therefore in its contained phytin rather than in the fiber.

Shortly after the previous publication, Mendel and Underhill (3) reported on the physiological action of phytin in the dog and the rabbit. They found that the compound was readily absorbed and had no characteristic effects upon the health of the animals. However, the phosphorous of the phytin in both the dog and the rabbit was almost entirely eliminated through the kidneys rather than the feces—quite the opposite of the cow but similar to the situation in man. They thought that this mode of excretion of the phytin-phosphorous might be intimately related to its laxative action, for in the dog and rabbit purgation could not be constantly provoked. If the phytin-phosphorous had been excreted by the intestine, then, perhaps, it would have formed inorganic phosphorous compounds (e.g., sodium phosphate) which might then have acted as a laxative.

In 1909, Hart, McCollum, and Humphrey (4) studied the question further, using the cow as an experimental animal. They confirmed the earlier work showing the laxative effect of phytin in the cow, furthermore maintaining that in this animal crude fiber had but little effect in relieving constipation. They also noted that "when the washed bran ration was supplemented with a quantity of magnesium and potassium as chlorides and sulphates, equivalent to these bases removed in the washing process, a sudden change from the whole bran to the washed bran ration could be made without in any way modifying the character of the feces which

remained normal." This again tended to support the hypothesis of Mendel and Underhill that phytin owed its cathartic nature to laxative mineral compounds that were formed in its katabolism and were excreted through the gut.

The latest report dealing with the subject is that of Williams (5). Dogs were used as the subjects of the experiments. They were fed a synthetic control diet, made according to Cowgill's method (6), to which were added varying amounts of crude fiber or other test objects. The number of defecations and the total weight of dried feces in each metabolism period were used as the criteria of laxation. The washed bran used in these experiments was prepared by washing it with cold water until it gave negative tests with iodine. The bran was then dried and used. It should be pointed out that washing bran with water alone will not remove all of the phytin. Averill and King (7) have shown that extraction with water alone causes wheat bran to lose about half of its phytin content but not much more. The washed bran used by Williams had a content of crude fiber of 17.24 per cent; of ash 3.12 per cent; and of pentosans, 36.94 per cent. He found that washed bran possessed a markedly laxative effect and that most of this laxative effect could be produced by the crude fiber of the bran.

The chemical nature of phytin has been most thoroughly investigated by R. J. Anderson (8). He concludes that the phytin of bran is probably inosite hexaphosphate,  $C_6H_{18}O_{24}P_6$ . Naturally occurring phytin is mainly a mixed calcium, magnesium, and potassium salt of inosite hexaphosphate.

The phytin content of food stuffs has been studied by Averill and King (*loc. cit.*) and by E. Arbenz (9). Both find the phytin content of wheat bran to be 4.5 per cent.

#### EXPERIMENTAL PROCEDURE

To investigate this subject more fully a series of experiments was performed using the white rat. Special metabolism cages were constructed in such fashion that the feces were separated from the rat as soon as voided. (See Figs. 1 and 2). Non-scattering diet cups were used. The feces were collected every 24 hours and were placed in a drying oven (kept at 70° C). It was not feasible to measure the output of wet feces since considerable drying occurred at room temperature. The weight of the dry feces for the total experimental period was then determined after the feces had come to constant weight in the drying oven. The beginning and end of each feeding period was determined by carmine which usually took from 18 to 24 hours for appearance.

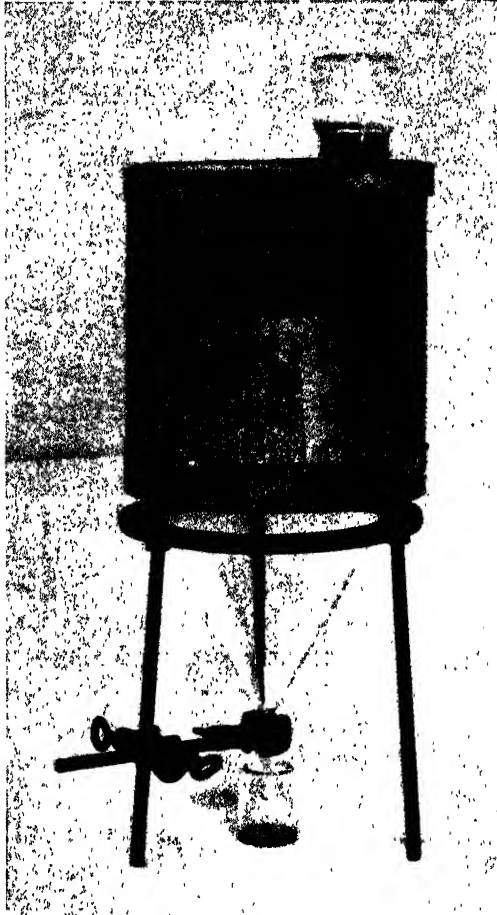


FIG. 1.—Metabolism cage for collection of rat feces, assembled

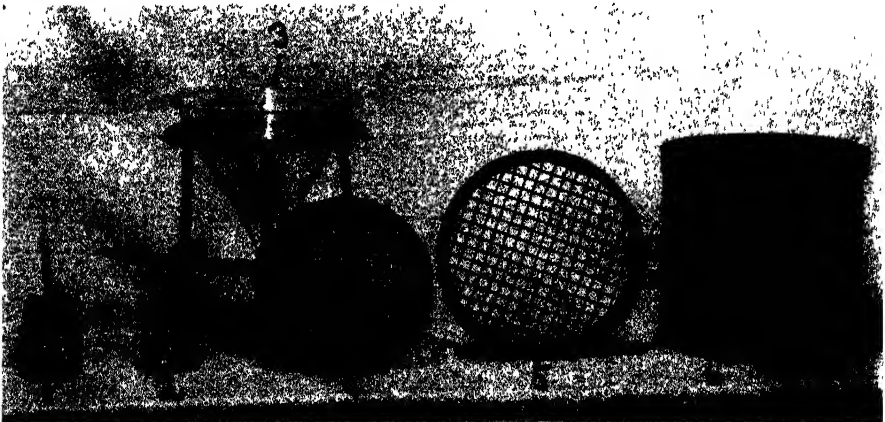


FIG. 2.—Component parts of metabolism cage for collection of rat feces.  
Description of parts at bottom of page opposite.

In the first experiment, a group of rats was fed a basic diet which contained no bran, fiber, phytin, or bran-ash. In subsequent experiments varying amounts of these substances were added to the basic diet to determine the effect on laxation.

Two basic diets were used differing only in the source of their protein—in the one casein, in the other dried beef powder. It was found that the consumption of the casein diet was not sufficient in many cases to maintain the weight of the adult rats used but the beef diet was satisfactory in that regard. The basic casein and beef diets consisted of the following:

Casein.....	18.0	Dried Beef Powder.....	15.0
Starch.....	53.0	Starch.....	61.0
Salt mixture (Osborne & Mendel)....	4.0	Salt mixture.....	4.0
Lard.....	20.0	Lard.....	17.0
Cod liver oil.....	3.0	Cod liver oil.....	3.0
Yeast concentrate <sup>1</sup> .....	2.0		
		plus daily vitamin B tablet	100.0
	100.0		

The results of feeding these diets to a group of 6 rats are summarized in Table I.

Table I shows that the 6 rats on the basal casein diet excreted an average of 7 stools daily with a total dry weight of 258 mg. and individual weights of 38 mg. The 2 rats on the basal beef diet excreted an average of 3.8 stools daily with a total dry weight of 220 mg. and an individual weight of 59 mg.

Three criteria of laxation may be used with the rat: 1. The daily number of stools, 2. The dry weight of each stool, 3. The total dry weight of stool produced daily. Hence one may observe a double action of laxatives; 1.—in increasing the number of stools, 2.—in increasing the weight of each stool. Either of these independently may increase the total daily weight of the feces and this separate effect was observed.

1. Three ounce wide-mouthed bottle with drinking tube six inches long and 5/16 in. in diameter.

2. Non-scattering food cup consisting of,

a. 200 c.c. tall beaker, 3½ in. X 2½ in.

b. wide-mouthed 2 oz. bottle with mouth 1 3/16 in. wide.

3. Glass funnel, 8 in. dia.

4. Wire screen, 6¼ in. dia., 1/16 in. mesh.

5. Wire screen, 8¾ in. dia., ¼ in. mesh.

6. Galvanized iron cage, 8¾ in. dia.

7. Cup holder.

<sup>1</sup> "Vegax."

It will be noted that half of the rats on the basal casein diets lost considerable amounts of weight probably due to their failure to ingest sufficient amount of the diet. It is possible that their stool production was somewhat subnormal but not sufficiently so to invalidate their use

TABLE I  
THE EFFECT ON FECAL PRODUCTION OF BASIC CASEIN AND BEEF DIETS

Rat No.	No. days on diet	Change in rat wt. grams	Diet* grams	Dry stool* mg.	No. of stools*	Av. wt. ea. stool mg.	Type of stool	Type of diet
1.	9	-20	4.7	238	6.0	40	small	casein
2.	9	-40	3.2	203	7.6	27	"	"
3.	9	-3	6.55	245	5.6	44	"	"
4.	9	-20	5.0	264	8.7	30	"	"
5.	9	-2	6.9	300	8.9	34	"	"
6.	9	+1	6.35	300	5.5	55	"	"
Av. of casein series		-14	5.78	258	7.0	38	small	
4.	5	+4	5.2	216	4.4	49	"	beef
5.	5	+6	4.4	224	3.2	70	"	"
Av. of beef series		+5	4.8	220	3.8	59	small	

\* Daily averages.

in determining the average. If we arrange the 6 casein rats according to the daily grams of diet ingested, the following series may be constructed.

Rat no.	Daily diet in grams.	Av. Total daily stool in mg.	Daily no. stools	Wt. each stool in mg.
2.....	3.2	203	7.6	27
1.....	4.7	238	6.0	40
4.....	5.0	264	8.7	30
6.....	6.35	300	5.5	55
3.....	6.55	245	5.6	44
5.....	6.9	300	8.9	34

In general the rats with higher food intake have a somewhat larger weight of daily stools but there is no constant effect either upon the number of stools or upon the weight of each stool.

After determining the stool production of rats upon the basal fiber-free diet, they were fed diets containing unwashed bran, phytin-free bran, bran fiber, bran ash, and phytin with the purpose of ascertaining to what constituent bran owed its laxative nature. The unwashed bran had a crude fiber content of 9 per cent as determined by the method of the Association of Official Agricultural Chemists (10). Its ash content was

6 per cent. Its phytin content was not determined, but analyses by Averill and King and by Arbenz (*loc. cit. supra*) show that the phytin percentage of bran is 4.5.

Two rats were fed a diet containing 2.5 per cent of bran. The diet consisted of the following:

Unwashed bran. . . . .	2.5
Casein. . . . .	18.0
Starch. . . . .	50.5
Salt mixture (O. & M.). . . . .	4.0
Cod liver oil. . . . .	3.0
Yeast concentrate. . . . .	2.0
Lard. . . . .	20.0
	<u>100.0</u>

Table II is a summary of the results.

TABLE II  
THE EFFECT ON FECAL PRODUCTION OF A DIET CONTAINING 2.5 PER CENT BRAN

Rat No.	No. days on diet	Change in rat wt. grams	Diet* grams	Bran* mg.	Fiber* mg.	Phytin* mg.	Ash* mg.	Dry* stool mg.	No. of stools*	Av. wt. of ea. stool mg.	Type of stool
1.	9	-10	6.8	170	15	7.5	10.2	329	7.5	44	small
2.	6	- 2	6.5	162	14.6	7.3	9.7	269	7.0	38	"
Averages			6.65	166	14.8	7.4	9.9	299	7.25	41	"

\* Daily averages.

It is thus seen that when rats eat bran at the average rate of 166 mg. daily there is little if any laxative effect. Both these rats weighed about 190 grams and one may therefore calculate that a daily ingestion of unwashed bran of 0.9 grams per kilogram of rat weight is without appreciable effect on laxation. On the other hand, the addition of the bran to the diet did increase its palatability to the rat for we find increased ingestion with slight gains in weight; upon the basal casein diet both these rats had lost weight markedly.

Unwashed bran was next fed to a group of 3 rats at a dietary level of 5 per cent. The diet consisted of the following:

Unwashed bran. . . . .	5.0
Casein. . . . .	18.0
Starch. . . . .	48.0
Salt mixture (O. & M.). . . . .	4.0
Lard. . . . .	20.0
Cod liver oil. . . . .	3.0
Yeast concentrate. . . . .	2.0
Total	<u>100.0</u>

The results of feeding the 5 per cent bran diet are summarized in Table III.

When bran is ingested at the rate of 300 mg. daily or over one begins to see its laxative effect. This effect is three-fold: 1.—There is an increased number of stools, 2.—there is an increased total weight of dried stools, and 3.—there is a slight increase in the size of each stool. This third result is better seen in the fresh stool than by considering the weight of the dry stool. The bran feces are apparently more moist than the normal feces and the increase in the weight of the individual stool would probably be more marked if one were to compare moist weights rather than dry weights. The increased moisture content of feces produced by feeding bran was also noted by Williams (5) in the dog.

It should be observed in connection with this feeding experiment that whereas the feeding of 0.9 gram of bran per kg. of rat was without laxative effect, the feeding of amounts above 1.42 gm. per kg. was definitely laxative. Both rats 5 and 6 (See Table III) ingested about 300 mg. of bran daily but rat 6 had a greater fecal output and ate 1.94 gm. of bran per kg. whereas rat 5 ate 1.42 gm. per kg. Rat 7 showed an enormous increase in all items, especially in daily number of stools, in total dried weight of stool, and in grams of bran per kilogram. The dry weight of the individual stool was but slightly affected.

To study the effects of bran at a high level of intake a diet was fed which contained 10 per cent of unwashed bran. This diet was composed of the following:

Unwashed bran . . . . .	10.0
Casein . . . . .	18.0
Starch . . . . .	43.0
Salt mixture (O. & M.) . . . . .	4.0
Lard . . . . .	20.0
Cod liver oil . . . . .	3.0
Yeast concentrate . . . . .	2.0
Total	100.0

It was fed to a group of 6 rats and the results are tabulated in Table IV. This table has been arranged according to the level of daily ingestion of bran so that, for example, the first rat (No. 6) has the lowest bran ingestion and the last rat (No. 7) has the highest. All the rats did well on this diet; there was an average gain of weight for the group of 10 grams. The daily level of bran ingestion ranged from 560 mg. to 850 mg. The total daily weight of dried stool was quite high and ranged from 520 to 870 mg. Furthermore, with the exception of rat 1, the weight of dried stool in-

TABLE III  
THE EFFECT ON FECAL PRODUCTION OF A DIET CONTAINING 5 PER CENT BRAN

Rat No.	No. days on diet	Change in rat wt. gm.	Diet* gm.	Bran* mg.	Fiber* mg.	Phytin* mg.	Ash* mg.	No. of stools*	Type	Dry stool* mg.	Wt. each* mg.	Wt. rat gm.	Bran per kg. gm.
5.	9	+ 4	5.9	295	26.5	13.3	17.4	7.8	med.	312	40	208	1.4
6.	9	+ 1	6.2	310	27.9	14.0	18.6	7.1	"	430	61	160	1.9
7.	6	+20	12.1	610	55.0	27.5	36.6	14.6	"	810	56	220	2.8
Averages			8.08	405	36.4	18.2	24.2	9.8	"	517	52		2.0

\* Daily averages.

TABLE IV  
THE EFFECT ON FECAL PRODUCTION OF A DIET CONTAINING 10 PER CENT BRAN

Rat No.	Days on diet	Rat wt. gm.	Change in wt. gm.	Diet* gm.	Bran* mg.	Fiber* mg.	Phytin* mg.	Ash* mg.	Dry stool* mg.	No. of stools*	Av. wt. each mg.	Type	Bran per kg. gm.
6.	10	154	- 4	5.6	560	50.3	25.1	33.0	520	8.5	61	med.	3.3
1.	10	205	+ 6	6.6	660	59.0	29.5	39.6	870	9.6	91	large	3.2
5.	10	214	+ 8	6.7	670	60.3	30.1	40.2	560	7.8	72	med.	3.1
8.	10	247	- 3	8.2	820	74.0	37.0	49.2	665	14.7	45	"	3.3
4.	7	165	+22	8.3	830	74.5	37.4	49.8	760	14.8	51	small	5.0
7.	10	237	+10	8.5	850	76.5	38.2	50.9	753	13.3	57	med.	3.6
Averages				7.3	730	65.7	32.8	43.8	688	11.4	60		3.6

\* Daily averages.



creased proportionally with the increase in dietary bran. The number of stools was markedly increased and the weight of the individual stool slightly increased. Bran, therefore, in causing laxation does so both by increasing the number of stools and the size of each stool. It cannot be said which is more affected since the wet weight of the stool is unknown. The bran ingestion calculated on the basis of kilogram of rat weight is fairly constant, ranging from 3.1 to 3.6 grams with the exception of rat 4 which ate such large amounts of diet that a gain in body weight of 22 grams resulted.

To bring out the effects of bran in a quantitative fashion, a table was constructed in which the percentage of bran in the diet was ignored but the actual milligrams ingested were progressively set down. In Table V one may therefore determine the probable stool production at a given level of bran ingestion. The averages of the basal casein diet are included for comparison. The laxative effect of bran begins when 300 mg. or more are ingested daily or at a level of about 2 grams per kg. of rat weight. The proportionality between bran ingested and fecal production is clearly shown. The two rats marked with an asterisk are the only exceptions to the general trend.

TABLE V  
RELATION BETWEEN BRAN INGESTION AND FECAL PRODUCTION

Rat No.	Av. Daily bran mg.	Daily dry stool mg.	Av. Daily no. of stools	Bran per kg. gm.
Casein average	0	258	7.0	0.0
2	162	269	7.0	0.9
1	170	329	7.5	0.9
5	295	312	7.8	1.4
6	310	430	7.1	1.9
6	560	520	8.5	3.3
7*	610	810	14.6	2.8
1*	660	870	9.6	3.2
5	670	560	7.8	3.1
8	820	665	14.7	3.3
4	830	760	14.8	5.0
7	850	753	13.3	3.6

We may conclude that bran quantitatively increases fecal production. What element in the bran is responsible for the increase?

Reverting to Table IV, one notes that the group of animals in which bran was most laxative were ingesting from 50 to 60 mg. of bran crude

fiber daily, 25 to 33 mg. of phytin, and from 33 to 44 mg. of bran ash. All of these substances increased or decreased exactly as the bran intake. Were they responsible for its laxative effect? The following types of diets were fed to answer this question:

1. A diet containing bran which had been rendered phytin-free and low in ash content; 2. A basal casein or beef diet to which varying amounts of the crude fiber of bran were added; 3. A basal diet to which phytin was added; 4. A basal diet to which bran ash was added.

#### THE EFFECT OF FEEDING PHYTIN-FREE, LOW ASH BRAN

Bran which was free of phytin and very low in ash content was prepared as follows:

1. Unwashed bran was extracted in 2 per cent HCl for six hours. 2. The bran was washed thoroughly with distilled water. 3. The bran was dried in the oven at 50°C. to a constant weight. 4. It was then dried in room air for 24 hours.

This air-dried bran had an ash content of 0.4 per cent. Its crude fiber and pentosan content were not determined but these substances must have been increased in percentage at least by the amount of water-soluble materials that were lost, the soluble carbohydrates, phytin, and ash. Any laxative effect of phytin-free, low-ash bran would therefore owe its potency to something other than phytin or ash.

Table VI is a tabulation of the results of a diet containing 10 per cent of phytin-free bran prepared as indicated above. The diet consisted of the following:

Phytin-free bran.....	10.0
Casein.....	18.0
Starch.....	43.0
Salt mixture (O. & M.)..	4.0
Lard.....	20.0
Cod liver oil.....	3.0
Yeast concentrate.....	2.0
Total	100.0

The table is arranged in order of increasing ingestion of bran. Several conclusions may be drawn with regard to the effect of ingesting the phytin-free diet. First, all the animals ate less of this diet than of the diet containing the unwashed bran and in fact ate so much less that they all lost weight. Evidently washing bran with 2 per cent HCl decreases its palatability. Secondly, there is a clear proportionality between the total weight of dry stool and the amount of bran ingested; rat 1 is the only

exception to the trend. Thirdly, a diet containing no phytin and far less bran ash than any previously fed proves to be markedly laxative. The average total weight of daily stools for the group is 674 mg. as compared with 688 for the group receiving 10 per cent unwashed bran. Moreover the stools are increased in size and number to the same degree.

One may state unequivocally that the phytin of bran plays no part in producing laxation in the rat. Similarly, the ash of bran is of little or no importance. The phytin-free bran does, however, contain a very high percentage of both crude fiber and pentosans since the extraction with dilute hydrochloric has removed most of the soluble substances. While the crude fiber was not estimated it may be calculated that the fiber content of the phytin-free bran was increased to at least 19 per cent. The extraction removed 4.5 per cent of contained phytin and 5.6 per cent of contained minerals. Therefore the crude fiber content of unwashed bran of 9 per cent must have been increased to at least 19 per cent. The pentosans were not determined. Williams (*loc. cit.*) has found that washed bran has a pentosan content of 36.94 per cent. Thus pentosans and crude fiber amounted to over 50 per cent of the phytin-free bran.

TABLE VI  
THE EFFECT ON FECAL PRODUCTION OF A DIET CONTAINING 10 PER CENT OF PHYTIN-FREE BRAN

Rat No.	Days on diet	Rat wt. gm.	Change in wt. gm.	Diet* gm.	Bran* mg.	Fiber* mg.	Ash* mg.	Dry stool* mg.	No. of stools*	Av. wt. each mg.	Type	Bran per kg. gm.
6.	9	143	-18	4.0	400	80**	1.6	470	8.5	55	med.	2.8
4.	9	174	-3	4.45	445	89	1.8	507	8.0	63	large	2.6
5.	9	208	-20	4.8	480	96	1.9	610	11.3	54	med.	2.2
1.	9	197	-21	5.8	580	116	2.3	818	7.8	105	large	2.9
7.	9	237	-10	7.35	735	147	2.9	759	13.3	57	large	3.1
8.	9	240	-10	7.55	755	151	3.0	881	17.0	52	med.	3.1
Averages			-14	5.65	565	113	2.3	674	11.0	61		2.8

\* Daily Averages. \*\* Minimum Values.

If bran owes its laxative effect to the unabsorbed food residues arising from crude fiber and pentosan, then one would expect a strikingly laxative effect from crude fiber fed in amounts equivalent to the combined pentosans and crude fiber.

To test this deduction, a group of 7 rats was fed varying amounts of crude fiber incorporated in the basal beef diet. The results are exhibited in Table VII. The table is again arranged according to the average amount of fiber ingested daily.

TABLE VII  
THE EFFECT OF CRUDE FIBER OF BRAN ON FECAL PRODUCTION

Rat No.	Days on diet	Rat wt. gm.	Change in wt. gm.	Crude fiber* mg.	Dry stool* mg.	No. of stools*	Av. wt. ea. stool mg.	Crude fiber kg. gm.
7	8	224	-16	45	275	5.6	49	0.20
8	8	224	-23	48	275	7.0	39	0.21
9	6	218	+12	76	529	12.0	44	0.35
14	6	161	+18	162	523	15.0	35	1.01
15	6	140	+14	180	610	17.0	35	1.29
11	3	170	+ 4	570	811	18.0	45	3.34

\* Daily averages.

The crude fiber used in this experiment was obtained from bran by the following method:

1. Unwashed bran was extracted with ether overnight to remove the fat.
2. The ether was decanted and the bran dried.
3. The bran was boiled for 30 minutes with 1.25 per cent sulphuric acid solution in a large round-bottomed flask to which was attached a reflux condenser.
4. The acid mixture was strained through cheese cloth and washed with hot water until neutral to litmus.
5. The residue was returned to the flask and boiled for 30 minutes with 1.25 per cent sodium hydroxide solution.
6. The straining through cheese cloth and washing with water was repeated.
7. The residue was dried to constant weight at 90° C. This method of obtaining crude fiber is closely similar to the standard methods for determining crude fiber. It does not include pentosans and the value given before for the crude fiber of unwashed bran (9 per cent) does not include pentosans. Crude fiber is essentially cellulose and the pentosans are the hemicelluloses, which require a different method for determination.

Table VII shows that with increasing concentrations of crude fiber there is increasing laxation. Interestingly enough, the crude fiber of bran produces an increased number of stools which in the moist state re-

semble the bran feces but when dry show no increased individual weight above normal.

While it has been shown that the absence of phytin or of bran ash does not in any way lessen the effectiveness of bran as a laxative, the question arises whether these substances may not have some inherent laxative effect. This was investigated by feeding to a group of 5 rats, phytin and ash at concentrations even higher than are present in the 10 per cent unwashed bran diet. Referring to Table IV, it will be seen that in this diet the average ingestion of bran ash was 43.8 mg. daily and of phytin 32.8 mg. daily. The following diets were fed:

1.-2 per cent phytin, 2.-0.6 per cent bran ash, 3.-1.2 per cent bran ash. The composition of the first diet was as follows:

Phytin.....	2.0	
Casein.....	18.0	
Starch.....	53.0	
Salt mixture.....	4.0	
Lard.....	20.0	
Cod liver oil.....	3.0	
		<hr/>
plus daily vitamin B tablet	100.0	

The bran ash diets were composed as follows:

Bran ash.....	0.6	1.2
Dried powdered beef.....	15.0	15.0
Starch.....	60.4	59.8
Salt mixture.....	4.0	4.0
Lard.....	17.0	17.0
Cod liver oil..	3.0	3.0
		<hr/>
plus daily vitamin B tablet	100.0	100.0

The phytin was prepared from bran by the method of Clark (11): Two and one-half pounds of bran were extracted for five hours with 2.0 per cent hydrochloric acid at a room temperature of about 20°C. The extract was filtered through cheese cloth and allowed to settle overnight. The clear, supernatant liquid was siphoned off, heated to boiling to coagulate the protein, and allowed to cool. After settling, the clear amber-colored liquid was again siphoned off from the residue, and heated to boiling. Ammonia was added until the solution was just alkaline and the boiling continued for a short time. A large quantity of a flocculent precipitate separated out. This was filtered while the solution was still hot, and was washed with boiling water. This brown, sticky precipitate was extracted with two liters of 8.0 per cent acetic acid, and filtered from an insoluble residue. The amber-colored solution thus obtained was heated to boiling, and a large

amount of phytin separated out as a fine, white precipitate which redissolved on cooling except for a slight insoluble residue which was filtered off. The clear filtrate was diluted with an equal volume of water and heated to boiling, and again made alkaline with ammonia. A large, white precipitate settled out, which was again filtered while hot, and washed thoroughly with boiling water. This white precipitate was extracted with the smallest possible amount of 0.8 per cent acetic acid, and filtered from a slight insoluble residue. This acetic acid solution was heated to boiling, and the phytin separated out as a heavy white, powdery precipitate, which was filtered off while hot, leaving in the filtrate all inorganic phosphates which were soluble in the acetic acid solution. This precipitate was thoroughly washed with hot water, alcohol, and ether, and dried in the air at room temperature.

Bran ash was prepared by ashing large quantities of unwashed bran in large silica dishes over open burners. After the ashing was completed the residue was finely powdered and incorporated in the diet.

Table VIII summarizes the results with phytin and bran ash. Phytin is without laxative effect when ingested at the rate of 72 to 84 mg. daily. This is over twice the amount ingested by the animals in the 10 per cent unwashed bran diet. Bran ash is without effect when eaten at a level of 48 to 66 mg. daily. This is slightly higher than the average ingestion of the 10 per cent bran group. When the amount ingested is increased to about 100 mg. daily there is a slight laxative effect in one of the rats (No. 13) and none in the other (Rat 12).

TABLE VIII  
THE EFFECT OF PHYTIN AND BRAN ASH UPON FECAL PRODUCTION

Type of diet	Rat No.	Days on diet	Change in rat wt. gm.	Diet* gm.	Phytin* mg.	Ash* mg.	Dry stool* mg.	No. of stools*	Av. wt. ea. stool mg.	Type of stool
2% phytin	7	5	-16	3.6	72	0	220	5.0	43	small
2% "	8	5	-4	4.2	94	0	302	7.6	40	"
0.6% ash	10	4	+10	7.8	0	48	232	7.8	30	"
0.6% "	11	6	+14	9.8	0	66	116	7.0	17	"
1.2% "	12	4	+12	7.8	0	94	251	7.7	32	"
1.2% "	13	6	+16	9.8	0	118	390	9.0	43	"

\* Daily averages.

It is not surprising that bran ash in very high concentrations should have some laxative effects because of its contained magnesium and sulphur salts. However, the effect is small and not constant.

## SUMMARY AND CONCLUSIONS

1. A method of studying the fecal output of the rat is described. In the rat:
2. Bran acts as a laxative in a quantitative fashion—increasing amounts of bran produce increasing weights of feces.
3. Phytin-free bran has lost none of the laxative qualities of bran.
4. The ash of bran and the phytin contained in bran are not laxative in the quantities in which they are contained in bran. Bran ash fed separately may be slightly laxative in high concentrations.
5. Bran owes its laxative action to the crude fiber and pentosans which it contains.

## REFERENCES

1. Jordan, W. H., E. P. Patten, and A. J. Hart., A Study of the Metabolism and Physiological Effects of Certain Phosphorous Compounds with Milch Cows. *Amer. Jour. Physiol.*, 1906, XVI, 269.
2. Sherman, H. C., Chemistry of Food and Nutrition. New York, 1926.
3. Mendel, L. B., and F. P. Underhill, Experiments on the Physiological Action and Metabolism of Anhydro-Oxymethylene-Di-phosphoric Acid (Phytin Acid). *Amer. Jour. Physiol.*, 1906-07, XVII, 88.
4. Hart, E. B., E. V. McCollum, and G. C. Humphrey, Role of the Ash Constituents of Wheat Bran in the Metabolism of Herbivora. *Amer. Jour. Physiol.*, 1909, XXIV, 86.
5. Williams, G. A., A study of the Laxative Action of Wheat Bran. *Amer. Jour. Physiol.*, 1927, LXXXIII, 1.
6. Cowgill, G. R., *Jour. Biol. Chem.*, 1923, LVI, 725.
7. Averill, H. P., and C. G. King, Phytin Content of Foodstuffs. *Amer. Chem. Soc. Jour.*, 1926, XLVIII, 724.
8. Anderson, R. F., *N. Y. Agric. Exp. Sta., Tech. Bull.* No. 1-41; 1906-1915. *Tech. Bull.* No. 22; *Jour. Biol. Chem.*, 1915, XX, 493.
9. Arbenz, E., *Mitt. Lebens m. für Hyg.*, 1922, XIII, 45.
10. Methods of Analysis of the Assoc. of Off. Agric. Chemists. Washington, 1925, p. 117.
11. Clark, George, *Jour. Chem. Soc.*, 1914, CV, 535.



## Editorial Review

### THE EMPTYING MECHANISM OF THE STOMACH\*

THE first important contribution to the physiology of the alimentary tract made on this continent was Beaumont's (1833) remarkable study of the stomach of Alexis St. Martin. To commemorate this achievement a *fac simile* copy of Beaumont's book, containing also Osler's (1902) well-known address on Beaumont as an introduction, was presented to each member of the recent International Congress of Physiologists at Boston. Beaumont's book appeared in 1833 and one of the four periods covered by his observations began in August 1829 and continued until March 1831. This issue of the Journal therefore falls within the 100th anniversary of Beaumont's labors. It is for this reason that this discussion appropriately begins with a brief characterization of this first American contribution.

A very brief allusion to the circumstances which gave Beaumont, an obscure Army surgeon, his great opportunity must suffice; for every text book of physiology in the past hundred years has made some reference to the case. St. Martin, a youth of 18, was wounded by the accidental discharge, at very close range, of a shot gun loaded with duck shot. The charge entered his body posteriorly, traversed the lower lobe of the left lung, tore away a piece of the diaphragm, perforated the stomach, and passed out through the abdominal wall just below the costal margin and only two inches below the left nipple on a line from the nipple to the crest of the ilium on the same side. A large, lacerating wound was left. Beaumont, then stationed at Michilimackinac, where the accident occurred, reached the boy within 25 minutes, dressed his wound, and though despairing of his recovery, took him into his family later and personally nursed him back to health. Only after two years was the young man able to work. Beaumont began his observations in 1825, nearly three years after the accident, and took the subject with him to various army posts where he was stationed and kept up his experiments as best he could with a rather stupid and at times recalcitrant individual for the next eight years.

Osler, in the address to which reference has been made, quotes the following lines from Combe, a contemporary professor at Edinburgh, to indicate the value of Beaumont's work. "It would be difficult to point out any observer who excells him in devotion to truth and freedom from the trammels of theory or prejudice. He tells plainly what he saw and leaves every one to draw his own inferences, or where he lays down conclusions

\* Revised from an address delivered before The Philadelphia County Medical Society, Phila., Pa., on November 26, 1929.



he does so with a degree of modesty and fairness of which few perhaps in his circumstances would have been capable."

To give an idea of the state of knowledge, or rather lack of knowledge, of digestion, at this time, Osler refers to Dunglison who, in his "Work on Human Physiology" (published the very year of Beaumont's book) after discussing all the old theories regarding chymification of the food, quotes Wm. Hunter's witty remark—"Some physiologists will have it that the stomach is a mill, others that it is a fermenting vat, others again that it is a stew pan; but in my view of the matter, it is neither a mill nor a fermenting vat, nor a stew pan; but a stomach, gentlemen, a stomach."

For a critical account of Beaumont's work, just what light he was able to shed and what he left in obscurity, reference may be made to a 32-page review and critique of Beaumont's book by D. Francis Condie of Philadelphia, published the next year (1834) after its appearance, in the 14th volume of the American Journal of the Medical Sciences. Condie quotes from Raynier who published a work "*De Digestione in Ventriculo*" in 1792. "To arrive at anything like positive conclusions in regard to this subject, the experimenter must be enabled to inspect the interior of the healthy stomach whilst its functions are going on, and study there the modifications which the composition of the alimentary bolus undergoes from its entrance through the cardia until its final escape through the pylorus—we shall then, but I fear not before, be able to say what is the nature of digestion, and what are the powers by which it is effected." Condie continues, "the opportunity here required, the occurrence of which was no doubt thought impossible by the writer just quoted, has actually been furnished to Dr. Beaumont. By a surgical case nearly unique in its results, the interior of the stomach in a state of health, and in the perfect performance of its functions, has been laid open to his view, and he has been able to study daily for a series of years, the actions of that important organ—to mark the successive changes produced in the food during the process of digestion and to determine with accuracy the composition and properties of the gastric fluids, and their effects upon the different kinds of aliment in ordinary use . . . . The report of his experiments and observations constitutes unquestionably, in many particulars, the most important work ever published on the physiology of digestion." Condie evidently was familiar with the scientific literature of the time, and did not hesitate to point out wherein Beaumont's observations were incomplete and he regrets that the peculiar advantages possessed by Dr. Beaumont for studying the process of digestion did not fall to the lot of some one better trained than he, particularly in chemistry. The two most important problems; in what does chymifica-

tion consist? and in what manner is it effected? Condie thinks Beaumont left still pretty much in obscurity.

That the gastric juice really has a solvent power over the most insoluble material, however, Beaumont proved in the most conclusive manner. He determined the period of time required for pure gastric juice drawn from the stomach to reduce different alimentary substances to a paste, when kept at 100°F outside the body. It is from Beaumont that we derive our school book teachings regarding the relation of fiber and toughness in food to digestibility. The articles requiring longest time for chymification were found to contain much fatty or oily material and his statement that a slight admixture of bile aids digestibility of fatty foods to some extent, has a very modern sound.

Beaumont apparently was considerably mystified by the movements of the stomach. He is quite clear, however, on three points; one, that by alternate contraction and relaxation of the transverse muscular fibers a peristaltic motion is produced, which commences soon after the food is received, and causes the latter to "revolve around the interior of the gastric cavity." The second is that old and new food in the stomach are mixed thoroughly by a sort of "churning" of the contents. And third, that from the very beginning of chymification—from the time food is received into the stomach, until that organ becomes empty—*portions of chyme are constantly passing into the duodenum*. It is the last of these statements which forms the topic of the present discussion.

As might have been expected, many of Beaumont's observations have been wholly confirmed by later and more critically controlled observations, some have been only partially confirmed, and some few have been refuted. Carlson (1916) had an opportunity of observing a man with a stomach fistula for a period of nearly four years. This case has been called "a second Alexis St. Martin." But there is this important difference. St. Martin could chew and swallow his food directly into the stomach. Carlsons' Mr. F. V. had made the tragic mistake while a boy of trying to swallow strong caustic alkali and as a consequence the oesophagus was completely sealed. The fistula was therefore a deliberately located and circumscribed affair instead of a gaping aperture accidentally caused and crudely, though adequately, reduced surgically, as in St. Martin's case.

Carlson says of the movements of the stomach, as seen by direct observation in F. V., "the picture revealed by the gastric cavity when the empty stomach is in a period of rhythmic contractions is interesting, but rather bewildering, and we have ceased to wonder how Beaumont complete-

ly failed to grasp the character of the stomach movements in digestion, as he relied mainly on direct inspection of the stomach of St. Martin."

Carlson's method, in addition to direct inspection, was to record the motions of the stomach by means of a thin-walled, rubber balloon introduced into the stomach and connected by air transmission to a recording device. He was interested chiefly in the motions of the empty stomach or the hunger contractions which had been re-identified as the cause of the sensations of hunger by Cannon and Washburn in 1912. Records obtained from Mr. V. show the effects of a sudden dilatation of the balloon by increased pressure. The distension caused a few strong contractions which in turn caused the hunger sensations. These were felt and recorded by the subject himself. This is the behavior of the wholly empty stomach and Carlson and his pupils have shown that these strong contractions of the empty viscera gradually emerge from a series of tonus waves which begin ordinarily within 20 to 30 minutes after eating. The exact time depends to some extent on the degree of filling and to some extent upon individual idiosyncrasy. In addition to these types of movement we have of course the so-called digestion peristalses which had been seen by Beaumont and which are easily demonstrated by the X-ray and the opaque meal.

Cannon who introduced (1898) the use of the Roentgen ray for study of the gastro-intestinal movements and first demonstrated the peristaltic character of the movements of the filled stomach, demonstrated also in the cat how sensitive these movements are to strong psychic states like fear and anger. Rogers and Hardt (1915) in Carlson's laboratory found that the hunger contractions of the empty stomach both of animals and man are more easily inhibited by chemical agents introduced into the stomach and acting on the mucosa than are movements of the same type in the filled stomach. The mucosa as a matter of fact never does, in the experience of Carlson's laboratory, induce a reaction of greater activity. Anything which can act through the mucosa alone always produces inhibition. The nervous mechanism by which this is accomplished is either the short-fibered intrinsic plexus, or a center in the medulla and the splanchnic nerve.

In this connection the action of alcohol is of interest. Most people who have had the experience often enough to recognize it, claim that a glass of beer or wine or a cocktail before a meal increases appetite and possibly the hunger sensation. We must bear in mind the difference between appetite, which is simply the psychic appreciation of food depending upon the general bodily condition, and even upon such extraneous matters as the behavior of the stock market, and hunger which is the local sensation caused

by contraction (cramp) of the stomach. Carlson himself, writing in 1916, confessed that a glass of beer at meal time seemed to "awaken or increase appetite" and Pavloff has recorded an instance in his own experience where a glass of wine seemed to initiate the sensation of hunger the very minute the wine reached the stomach. Carlson therefore, from his own and from wide inquiries amongst his friends, expected to find in Mr. V. and in himself and others who submitted to the balloon method of study, that the introduction of alcoholic drinks would increase gastric tonus and initiate the contractions which give rise to hunger. To his surprise, the effect was just the opposite. Wine, beer, brandy, pure alcohol (diluted) introduced directly into the stomach always inhibited hunger contractions. If there is ever augmentation of appetite it must be through general effects on the nervous system rather than through contractions of the stomach.

Cannon's (1898) demonstration for the cat that the peristaltic wave, starting well up on the fundus of the stomach runs all the way to the pylorus, without interruption, was speedily confirmed by Roux and Balthazard (1898) on frogs, dogs, and human subjects, these authors also working with the X-ray. Greatly improved technique has substantiated this fact for the human stomach many times. Indeed, it is an every day observation now in every roentgenological laboratory. Opinion has fluctuated extensively, however, regarding two points in connection with the emptying of the stomach, one, the significance of the antrum and two, the control of the pylorus. Many roentgenologists have maintained, as did Hofmeister and Schütz (1885) from direct observation on the dog, and indeed, as Beaumont himself seems to imply quite clearly, that at times the transverse band completely pinches off the antrum, dividing the stomach into two cavities. According to this view the function of the whole pyloric portion is mainly that of discharging the contents into the duodenum. If, however, the transverse band does not pinch off completely, the pyloric portion has a triturating and mixing function as well as that of expulsion. Motion pictures of the dog's stomach exposed in a bath of salt solution, according to Alvarez and Zimmermann (1928), often clearly exhibit a systolic narrowing of the entire antrum. These authors do not find much evidence of a sphincter antri, or transverse band, but at times, the waves of contraction in the region of the incisura are deep enough to divide the stomach momentarily into two pouches. We shall encounter evidence presently that contractions of the antrum, even if serving a triturating function, are by no means random or ungoverned motions. Meantime let us see how the pylorus operates.

The whole conception of the stomach as a reservoir and feeder for

the intestine depends upon the behavior of the gateway. What governs the pylorus? Richerand ascribed to this mechanism something like intelligence when he said, "it has a peculiar tact which enables it to select from the contents of the stomach what is proper to pass through, while it holds back the remainder." With less teleology but more science, Hirsch (1893) and Serdjukow (1899), a pupil of Pavloff, both obtained evidence that an acid reaction in the duodenum retarded evacuation of the stomach. Tobler (1905) a little later found that a balloon put into the duodenum and inflated kept the pyloric sphincter contracted. Marbaix (1898) speaks of intestinal repletion as the governing factor. When the intestine has had enough it signals to the sphincter to shut off the supply. However, there is another factor involved; namely, the pressure exerted by the antrum on the stomach side. The roentgenological evidence is complete that peristaltic waves descend over the stomach continuously so long as there is food in the organ. Cannon (1898) thought his evidence was conclusively clear also that the pylorus does not open for every peristaltic wave passing over the antrum. When it does open, contents are discharged, but if the sphincter holds tight, the contractions of the antrum merely churn up the contents or squeeze some of it back into the fundus. Cannon (1904) had observed very different rates of evacuation of the stomach contents, depending on the kind of food ingested by his experimental animals. Carbohydrate meals emptied most rapidly, protein meals next and fatty meals most slowly. The behavior of the pylorus must in some way be related to the character of the food. The observations of Hirsch and of Serdjukow suggested to Cannon that the free acid of the stomach contents may not only close the pylorus when acting from the duodenal side of the sphincter, but may also give the signal for relaxation of the sphincter when acting from the stomach side, and thus Cannon built up his theory of the acid control of the pylorus. Carbohydrate does not bind up the acid of the gastric juice as does protein and thus we have an explanation of the more rapid passage of the former. The delay of a fatty meal can be made to fit into this theory, for fat retards the secretion of gastric juice and causes regurgitation of bile and pancreatic juice into the stomach, as Beaumont observed on St. Martin, thus neutralizing the acidity. Cannon marshalled many facts in support of this theory, but unfortunately there were then some facts and now there are many more for which it does not afford a satisfactory explanation (See *e.g.*, McClendon, 1915).

Cannon's complete theory was published in November 1907. It was not seriously challenged for about nine years. Spencer, Meyer, Rehfuess and Hawk reported in February 1916 that with the retention tube method they

could not obtain evidence that free HCl was necessary for opening of the pylorus, and Morse (1916) in October of the same year gave detailed measurements of discharge of water and acid of varying concentration from the stomach of dogs under ether, with the dorsal spinal cord destroyed so as to eliminate splanchnic inhibition. He found that neutral water left the stomach at a more rapid rate than any concentration of acid noted. In December 1916 Cole emphatically denied the whole conception, basing his contention on exhaustive roentgenological studies of the human subject with particular reference to the duodenal cap, which Cole believes should be regarded really as a part of the stomach. He sums up by saying "there is no roentgenological evidence in man of a periodic opening and closing of the pyloric valve independently of the gastric cycle, as described by Cannon." Cole's statement, that a meal composed of mixed foods begins to be evacuated often before it is completely eaten, has been many times confirmed. Water of neutral reaction has been observed through fistulae in dogs to pass in rhythmical spurts into the duodenum (Ivy, 1918) and a meal of bismuth and milk begins to pass the pylorus of the infant's stomach within five minutes of its ingestion (Pisek and LeWald, 1913). The acid control theory would offer poor comfort for the person with achlorhydria. If the gateway never opened except under the stimulus of free acid, there are a good many people living who would never get their food to the intestine. Ortner (1927) studied the evacuation rate in dogs having a Dastre cannula in the duodenum a few centimeters below the pylorus. He found that alkaline water was passed through sooner after ingestion than acid, and that strong acid (above 0.3 per cent HCl) distinctly inhibited the relaxation of the pyloric sphincter. Luckhardt, Phillips and Carlson (1919) prepared a dog so that chyme could be collected from the duodenum and also so that the movements of the antrum could be recorded by means of the balloon method. They found material issuing from the stomach with each tonus wave of the stomach wall. This was confirmed on a human subject also by recording the movements of the stomach, after a barium meal, by the balloon method and watching with the fluoroscope for the appearance of the duodenal cap. The cap appeared always coincidentally with the record of tonus rhythm which by fluoroscopic examination consisted of a plain succession of peristaltic waves.

An equally convincing study has been made by Wheelon and Thomas. Joseph and Meltzer (1911) had observed that with each contraction of the pyloric part of the stomach, the duodenum stopped its rhythmic activity and lost its tone, only to resume it again when the contractions passed off. Wheelon and Thomas (1922) in Joseph's department at St. Louis made a

more detailed study of this phenomenon. They invented an instrument, which they call an enterograph, consisting of three small rubber-covered chambers. It is introduced through a stomach fistula of a dog and so placed that one chamber lies in the duodenum, one in the pyloric sphincter and one in the antrum. Records are taken by air transmission through thick-walled small rubber tubes which likewise issue through the fistula. By this method they made numerous records which show clearly a well-defined coordination of events in antrum, sphincter and duodenum. When the antrum is relaxed the sphincter is always closed, and the duodenum is at least partially contracted. When the antrum is contracted the sphincter is relaxed and the duodenum is at least partly relaxed. This goes on with perfect regularity for hours. "The parts excited cranialward transmit caudalward to excite lower segments," and therefore the behavior of the three mechanisms falls into agreement with Bayliss and Starling's "law of the intestine," *i.e.*, a progressive band of constriction preceded by inhibition and followed by relaxation.

It would appear from these results of Wheelon and Thomas that free acidity can scarcely furnish the stimulus for opening of the pylorus. But they did not make direct observations on the chemical changes along with their study of the motor mechanism. This deficiency has just recently been supplied by James McCann working in the department of experimental surgery at the Mayo Foundation laboratories. McCann (1929a) was interested first in the question of the control of the acidity of the stomach. Boldyreff in 1907 proposed the theory of "the self-regulation of the acidity of the gastric juice" by regurgitation of alkaline duodenal fluids into the stomach. Among other facts he showed that when 200 cc. of 0.5 per cent HCl were introduced into the stomach of a dog, the acid was neutralized by regurgitation of duodenal alkalies, chiefly in the pancreatic juice. The rise of trypsin concentration in the stomach contents during the latter part of the fractional test meal by the retention-tube method of Einhorn and of Reh-fuss has been held by some clinicians to be in accord with Boldyreff's theory. McCann tested this hypothesis by eliminating the duodenal juices by draining them directly into the ileum. In other experiments he resected the antrum of the stomach entirely, thereby eliminating the alkaline mucous secretion of the antrum, to see whether it might play any part in the control of acidity. Seven dogs were subjected to the procedure of duodenal drainage, but fractional analyses of the stomach contents after this operation, and feeding with meat or stimulating gastric secretion with histamine, did not show any variations from the curves found before operation. Addition of carbohydrate and fat to the protein meal showed the same altera-

tions of the curves obtained by fractional analyses in operated dogs as in normal dogs. Regurgitation of alkali therefore is not an essential mechanism in the control of acidity. He concludes that the mechanism is essentially an intragastric one, and that the relationship between the concentration of free HCl and the rate of secretion agree with Pavloff's observations on gastric pouches. "It is a rule almost without exception," says Pavloff, "that the acidity of the juice is closely dependent on the rate of secretion; the more rapid the latter the more acid the juice, and *vice versa*." In McCann's dogs with the antrum resected there was increase in the rate of secretion or in the concentration of acid, such as is seen in normal dogs, indicating that the prepyloric segment is in some way concerned in the regulation of secretion.

In his second study McCann (1929b) correlated his method of fractional analysis of the gastric secretion with roentgenological studies of the motor phenomena. The curves of free acid, total acid and neutral chloride were plotted and roentgenograms taken at certain definite points on these curves. They show that the stomach begins to empty very soon after the meal is ingested and the rate of emptying increases progressively from early digestion until the process is complete, quite independently of free acid in the contents. "The emptying depends not on acidity but on progressive relaxation of the whole pyloric end of the stomach. The activity of this *pars pylorica*, of which the sphincter is merely the most efficient segment, depends on its irritability and, in these studies, on the stimulating action of raw protein to tonic and peristaltic action. With the disintegration of the protein to the products of digestion, there is a graded induction in the intensity of the stimulus to the antrum and consequently a progressive relaxation which permits more rapid evacuation."

Katsch (1927) of Frankfort has pointed out that we cannot consider the motor and secretory functions of the stomach as independent. Katsch's statement of stomach function in a single sentence is this, "to receive the food, hold it for a time, change it chemically and physically and, in proper rhythm with these changes, to pass the food along to the intestine." This conception, obtained from the clinic, is identical with that of McCann obtained from the experimental laboratory. Many of the gastro-intestinal disturbances have their origin in a breakdown of this nice coordination of movement to secretion and digestion. Too rapid evacuation may have identically the same consequences as too little secretion. It must be remembered too that the motility of the stomach has the purpose not merely to deliver the food to the intestine where the bulk of digestion takes place, but also to remove it from the stomach as rapidly as digestion takes place,



in order that digestion may further proceed. The accumulation of digestive products slows digestion just as the product of any mill slows up the mill unless removed promptly. The intestine gets rid of its products of digestion by absorption, but absorption does not take place in the stomach. The only means of getting rid of its products, partially digested only, as a rule, is to pass them along. Thirty years ago we thought of the churning, mixing motions as characteristic of the stomach and peristalsis as characteristic of the intestine. We must now reverse the order; peristalsis with regular rhythmic emptying is more characteristic of the stomach, while mixing, accomplished by rhythmic segmentation and peristalsis is more characteristic of the intestine. These conceptions were established mainly by X-ray studies on animals and on the human subject and the operative experimental work on animals has only recently caught up with it. The one method has the advantage of observing the relatively undisturbed organ; the other the advantage of direct approach by operation and direct chemical examination of contents. Both methods are essential.

Rehfuss and his collaborators (1919, 1920), particularly Hawk and Bergeim, using the retention tube method, have added much information to what we knew before regarding the digestibility of—or, as they prefer to call it, the “gastric response” to—different foods. In the course of this work they observed that stomachs differ greatly in their response to the same kind of food. Some respond promptly and decidedly to the entrance of food, whereas others respond slowly and indifferently. One type of stomach empties very quickly whereas a second type evacuates slowly under like dietary conditions. They grouped their subjects into the rapid-emptying and slow-emptying types and found, for example, with test meals of 100 grams of beef the average emptying time for the former was 2 hours and 35 minutes, for the latter 3 hours and 25 minutes. They do not make a particular point of it, but doubtless Rehfuss and Hawk would agree, that there are all sorts of gradations in these two classes, so that the real contribution which they made ten years ago is, that stomach-emptying time,\* like reaction time to sound, or any one of a dozen other physiological reactions which can be measured in time, is decidedly an *individual* matter. The rather extensive variability in the type of peristalsis seen in different animals, or even in the same animal at different times (Alvarez, 1928), probably affords sufficient basis for this individual gastric behavior.

Some clinics, as, for, example, that of Katsch at Frankfort, have now adopted instead of a test meal, some standard stimulant to the gastric

\* Mattill and Smith (1930) on another page of this number show curves of average evacuation rate in rats fed on different cereals.

glands like 20 per cent alcohol or pure caffeine. Katsch uses also a standard color dissolved in the stimulating solution. Two drops of 2 per cent methylene blue in 300 cc. of water containing two tenths of a gram of *cafeinum purum* he finds sufficient. For one half hour after introduction of the retention tube, he draws small samples every ten minutes in order to establish a base line of free acidity, total acidity, total chlorides and pepsin. Then the stimulating solution is introduced and with the tube still in place he draws a 10 cc. sample every ten minutes, compares the color with the original color of the solution and again determines in each sample the acidities, chlorides and pepsin. The color chosen enables him to detect any bile regurgitated. Katsch rightly objects to calling the resulting curves which are obtained in this way "secretion curves." They should be called acidity curves, chloride curves and pepsin curves; for even with pure solutions as stimuli what one draws up is never pure gastric juice, except after the test solution is completely removed, and even then one may find bile, pancreatic juice or saliva. To give a couple of examples only. There is a certain type of motor-compensated hypersecretion in which the stomach is excited to produce a great deal of juice; but a hypermotility removes it rapidly, so that only a small amount of content is found. Again a late-acid curve may be given in certain cases of gastritis where the secretory power is weak and in cases of partial stenosis of the pylorus the curve of acidity may be identical.

How then is the function of secretion related to the motility of the stomach? It will be necessary to state rather dogmatically the results of recent work. Pavloff's demonstration thirty years ago of the psychic secretion of gastric juice through the vagus and the demonstration by Babkin, one of Pavloff's most brilliant students, that the secretion of the stomach, like that of the salivary glands, may be educated to respond to a conditioned reflex, has brought the secretion of this organ, like its motility, definitely into the psychological realm. No longer can we doubt that both motor and secretory activities of the stomach are subject to a certain degree of control from the higher centers, for both motility and secretory phenomena, especially the conditioned reflex type of secretion, are affected by obliteration of the cerebral cortex in dogs. This central or reflex control of secretion, however, affects ordinarily only the first gush of gastric juice which occurs before the meal is taken or while it is being appreciated through the gustatory nerves. What regulates the flow of gastric juice after the meal is safely in the stomach? Following the demonstration by Bayliss and Starling of secretin as the hormone of the pancreas providing for the flow of that juice as long as acid chyme is coming into

the intestine, Edkins (1906) first demonstrated the presence of a similar hormone in the stomach, later named *gastrin* by Keeton. Ivy and Farrell (1925) have now placed the hormone control of the stomach beyond the shadow of a doubt by their transplantation experiment. Cutting off a little bag of stomach wall in the fundus with its blood supply, Ivy transplanted it into the mammary gland of a dog which had recently suckled a litter of pups. Waiting until a new blood supply was established from the mammary vessels to this little stomach, he then severed the original supply including any extrinsic nerves. A fistulous opening into the pouch enabled him to study the rate of secretion from its gastric glands. Whenever the dog was fed, the pouch secreted. The only possible connection between the stomach remaining in its normal position and the transplanted pouch was by way of the blood supply. The substance which stimulates (*i.e.*, gastrin) is formed whenever food reaches the pyloric portion of the stomach. It is formed by the gastric mucosa in that region, absorbed into the blood, and carried normally around through the general circulation and back to the fundic glands. In Ivy's experiment it is not certain that gastrin was the agent but something in the blood was carried also to the transplanted pouch and caused it to secrete. Various substances in the pyloric region, like extract of meat, products of protein digestion, soaps, lactic acid, butyric acid, pancreatic juice, bile, soda solution, dextrose, extracts of vegetables and alcohol, can stimulate the production of gastrin and thus indirectly stimulate the fundic glands where only HCl as well as pepsin is formed. Moreover, these same substances stimulate the production of gastrin, not so effectively, but demonstrably, when introduced into the duodenum (see Keeton and Koch, 1915). We thus have a provision for the continuous production of gastric juice after we have lost interest in the food from the psychological point of view.

The secretion of gastric juice therefore would seem to be related to the emptying movements of the stomach by having a common cause. McCann's recent work indicates, if it does not prove, that the emptying depends on irritability of the pyloric region and on the stimulating action of the food to produce tonic and peristaltic contraction. Food in this same region excites the production of gastrin and consequently of gastric juice. Demonstration of a direct relationship between the two phenomena is approached in the experiments of Ivy and Farrell (1926) in which the transplanted gastric pouch was shown not only to secrete when the stomach proper secreted, but also to contract when the stomach proper contracted and to rest when it rested. Does the common cause act on the two mechanisms by means of the same agency or only simultaneously? Babkin

(1928) has reported recently on some Russian work done in 1923 and 1924 which shows that extract of beef put into the circulation can stimulate intestinal movements and when placed in an isolated loop of intestine can cause movements in another loop with which the first is connected only by way of the blood. Is there a motor hormone produced in the pyloric region of the stomach by absorption of food or is the food itself, after absorption, the stimulating agency to produce relaxation of the pyloric musculature and increased tonus and peristalsis above?

Whatever may be the explanation, it is quite clear that modern work tends to confirm the statement of Beaumont that "from the time food is received into the stomach until that organ becomes empty, portions of chyme are constantly\* passing into the duodenum, through the pylorus." This takes place slowly at first, "but is rapidly accelerated towards the conclusion of digestion, when the whole mass becomes more or less chymified." "As the food becomes more and more changed from its crude to its chymified state, the acidity of the gastric fluids is considerably increased . . . and the general contractile force of the muscles of the stomach is augmented . . . giving the contained fluids an impulse toward the pylorus" (loc. cit. 113).

J.R.M.

#### BIBLIOGRAPHY

- Alvarez, W. C., 1928, The mechanics of the digestive tract. New York, 2nd ed.  
Alvarez, W. C. and Arnold Zimmermann, 1928, Movements of the stomach. *Amer. Jour. Physiol.*, LXXXIV, 261  
Babkin, B. P., 1928, The endogenous and exogenous chemical stimuli affecting the motility of the alimentary canal. *Canad. Med. Assoc. Jour.*, XVIII, 267.  
Beaumont, W., 1833, Experiments and observations on the gastric juice and the physiology of the stomach. Plattsburg, N. Y., reprinted, Boston, Mass. 1929.  
Bergeim, Olaf, M. E. Rehfuess and P. B. Hawk, *et al.* 1919, 1920, Gastric response to foods. *Amer. Jour. Physiol.*, XLVIII, 411; XLIX, 174, 204, 222, 254; LI, 332; LII, 1, 28; LIII, 54.  
Cannon, W. B., 1898, The movements of the stomach studied by means of the Röntgen rays. *Amer. Jour. Physiol.*, I, 359.  
Cannon, W. B., 1904, The passage of different foodstuffs from the stomach and through the small intestine. *Amer. Jour. Physiol.* XII, 387.  
Cannon, W. B., 1906, Recent advances in the physiology of the digestive organs. *Amer. Jour. Med. Sci.*, CXXXI, 563.  
Cannon, W. B., 1907, The acid control of the pylorus. *Amer. Jour. Physiol.*, XX, 283.  
Cannon, W. B., 1911, The mechanical factors of digestion. London and New York.  
Cannon and Washburn, 1912, An explanation of hunger. *Amer. Jour. Physiol.*, XXIX, 441.  
Carlson, A. J., 1916, The control of hunger in health and disease. Chicago.  
Cole, L. G., 1916, Motor phenomena of the stomach, pylorus and cap observed Roentgenologically. *Amer. Jour. Physiol.*, XLII, 618.

\* *i.e.*, as judged by a steady decrease in volume of stomach contents—Ed.

- Condie, D. F., 1834, Review of Beaumont's book. *Amer. Jour. Med. Sci.*, XIV, 117.
- Edkins, J. S., 1906, The chemical mechanism of gastric secretion. *Jour. Physiol.*, XXXIV, 133.
- Hofmeister, Fr. and E. Schütz, 1885, Ueber die automatische Bewegungen des Magens. *Arch. f. exper. Path. u. Pharm.*, XX, 1.
- Hirsch, A., 1893, Weitere Beiträge zur motorischen Funktion des Magens nach Versuchen an Hunden mit Darmfisteln. *Centralb. f. klin. Med.*, XIV, 377.
- Ivy, A. C., 1918, Contributions to the physiology of the stomach, XLVIII. Studies in water drinking. *Amer. Jour. Physiol.*, XLVI, 420.
- Ivy, A. C. and Farrell, J. I., 1925, Contributions to the physiology of gastric secretion, VIII. The proof of a humoral mechanism. *Amer. Jour. Physiol.*, LXXIV, 639.
- Ivy, A. C. and J. I. Farrell, 1926, Studies on the motility of the transplanted gastric pouch. *Amer. Jour. Physiol.*, LXXVI, 227.
- Joseph, D. R. and S. J. Meltzer, 1911, Inhibition of the duodenum coincident with the movements of the pyloric part of the stomach. *Amer. Jour. Physiol.*, XXVII, xxxi.
- Katsch, G., 1927, Pathologische Physiologie des Magensaftes und des Magenchemismus. *Handb. d. norm. u. path. Physiol.*, III, 1118.
- Keeton, R. W. and F. C. Koch, 1915, Distribution of gastrin in the body. *Amer. Jour. Physiol.*, XXXVII, 481.
- Luckhardt, A. B., H. T. Phillips and A. J. Carlson, 1919, Contributions to the physiology of the stomach, LI. The control of the pylorus. *Amer. Jour. Physiol.*, L, 57.
- McCann, J. C., 1929a, Studies on the control of the acidity of the gastric juice. *Amer. Jour. Physiol.*, LXXXIX, 483.
- McCann, J. C., 1929b, Studies on the emptying of the stomach. *Ibid.*, 497.
- McClendon, J. F., 1915, Acidity curves in the stomach and duodenum of adult and infants. *Amer. Jour. Physiol.*, XXXVIII, 191.
- Morse, W. E., 1916, The relation of acid to gastric discharge and duodenal regurgitation. *Amer. Jour. Physiol.*, XLI, 439.
- Ortner, A., 1917, Ein Beitrag zur Kenntnis der Magenentleerung und ihrer Beziehung zur Verdünnungssekretion des Magens. *Pflüger's Arch.*, CLXVIII, 124.
- Osler, W., 1902, William Beaumont, an address before the St. Louis Medical Society, Oct. 4. *Jour. Amer. Med. Assoc.*, XXXIX, 1223.
- Pisek, G. P. and L. T. Le Wald, 1913, The further study of the anatomy and physiology of the stomach based on serial roentgenograms. *Amer. Jour. Dis. Child.*, VI, 232.
- Reh fuss, M. E., Olaf Bergeim and P. B. Hawk, 1914, Gastro-intestinal studies, II. The fractional study of gastric digestion with a description of normal and pathological curves. *Jour. Amer. Med. Assoc.*, LXIII, 909.
- Rogers, F. T. and L. L. J. Hardt, 1915, The relation of the digestion contractions of the filled to the hunger contractions of the empty stomach. *Amer. Jour. Physiol.*, XXXVIII, 274.
- Roux, J. C. and V. Balthazard, 1898, Étude du fonctionnement moteur d'estomac, *Arch. de Physiol.*, 5 ser. X, 85.
- Serdjukow, A., 1899, Ueber die Bedingungen des Uebertrittes der Nahrung vom Magen in den Darm. Inaug. Dis., St. Petersburg. Abst. in *Maly's Jahresber.*, XXIX, 350; also under name of Serdinkow, A. S., in *Jahresb. d. Physiol.*, 1899, VIII, 214.
- Spencer, W. H., G. P. Meyer, M. E. Reh fuss and P. B. Hawk, 1916, Gastro-intestinal studies, XII. Direct evidence of duodenal regurgitation and its influence upon the chemistry and function of the normal human stomach. *Amer. Jour. Physiol.*, XXXIX, 459.
- Tobler, L., 1905, Ueber die Eiweißverdauung im Magen. *Zeitschr. f. physiol. Chem.*, XLV, 195.
- Wheelon, H. and J. E. Thomas, 1922, Observations on the motility of the duodenum and the relation of the duodenal activity to that of the *pars pylorica*. *Amer. Jour. Physiol.*, LIX, 72.

MARCH, 1930

STUDIES ON THE DESTRUCTION OF VITAMIN C  
IN THE BOILING OF MILK

BY E. W. SCHWARTZE, F. J. MURPHY AND R. M. HANN

*(From the Mellon Institute of Industrial Research,\* University  
of Pittsburgh, Pittsburgh, Pa.)*With the assistance of WILLIAM WAUGH, DEBORAH D. MARSH,  
MARY MARSH, and HELEN B. WIGMAN

Received for Publication—August 18, 1929

## INTRODUCTION

MILK always has been, and will undoubtedly continue to be, the most important and indispensable single food of man. Because of its importance in the dietary of the infant and growing child as well as the adult (1) its nutritive properties have been studied more than those of any other food. The present investigation is concerned with the degree of destruction of vitamin C during the boiling of this food. This is important to the public health, as the pediatrician usually feeds boiled cow's milk, when necessary to supplement or supplant breast feeding, for the purpose of obtaining satisfactory size of the particles of curd in the infant's stomach (2). It has been found that this is a most effective means of controlling the incidence of those alimentary infections known colloquially as "Summer Complaint" (3). With the development of the practice of boiling milk there has been considerable evidence advanced to show that ~~boiled~~ milk is not as rich in antiscorbutic properties as some other foods (1, 4). Although we would not care to depreciate the use of other foods containing high potencies of vitamin C, we would not pass lightly over the importance of milk as a source of this antiscorbutic vitamin, especially in view of its early reputation as the sole successful source of an antiscorbutic agent for infants.

Vitamin C was selected for study in our cooking experiments, since this appears to be the most unstable vitamin. The effects are clearly

\* Contribution from the Utensil Fellowship.

differentiated by the marked relative (if not absolute) difference in susceptibility of the rat and the guinea pig to its absence. Moreover, changes in academic thought are less likely to make abruptly obsolete the results of studies based upon such differences between two species of animals.

Hess and Fish (5) state that milk boiled for a short period does not induce scurvy so readily as pasteurized milk, although both practices lower the vitamin C content. Home pasteurization is less deleterious than industrial pasteurization, since the latter involves especially problems of aging. Evaporated milk ordinarily contains no vitamin C (6, 7) though sweetened condensed milk may contain it (4). Dried milk powder may contain it even in considerable amounts (4, 8). Hess (4) states that a pint of ordinary city milk a day per nursing infant would generally suffice, and this is within the regular feeding regimen. The fact that the infant at present is generally left to get his vitamin by "probability and chance," under conditions which are adverse to him, is sufficient to emphasize the marked possibilities of developing a milk supply sufficient in vitamin C for maintenance of health.

The fundamental factors operating to affect the vitamin C content of milk are; feeding practices of dairymen (9, 10, 11, 12), and temperature and exposure of the milk to oxygenation after it is produced (4), namely, storage practices, cooling, straining and pasteurization and repasteurization. Under the caption of oxidation *per se*, is also included the effect of the container coming in contact with the milk, because any effect this might have would probably be catalytic oxidation. Copper has been shown by Hess (13) to be particularly conducive to destroying vitamin C during the pasteurization process. Bright copper seems to be less liable to produce off-taste in milk when it is used immediately after cleaning (14), presumably since there is less oxide on the surface at this time. McCollum, Simmons, and Becker (15) have shown that ferrous sulfate added to the prepared type of rat food is conducive to the destruction of vitamin A.

The catalytic action of metals, especially those possessing more than one valency, is too well known in the field of chemistry to demand comment (16). Mellor (17) states that aluminum possesses only one valency. Moreover Waters (18) has shown that aluminum and zinc are inactive and suitable for containers for transformer oils which are subject to oxidation whereas some other metals are less suitable. It would appear possible, therefore, that aluminum might be shown to possess a distinct advantage as regards the absence of any oxidative effect on vitamin C. Its qualities in other respects for dairy equipment and comparisons with other metals are fully discussed by Hunziker, Cordes and Nissen (19).

### GENERAL DISCUSSION OF METHODS OF ASSAYING THE ANTISCORBUTIC VITAMIN

In the present state of our knowledge, vitamins are known only by their effects on animals. Their chemical isolation has not yet been accomplished and recent work on vitamin D and ultra-violet light illustrates that it is a debatable question whether they are entities or states of matter. Man, monkey, and the guinea pig, are the only animals, with the possible exception of the domestic pig, known to be susceptible to scurvy (4). Guinea pigs are used practically exclusively for studies on vitamin C. Whether vitamin C be simple or a complex mixture, this term refers merely to a difference in the response of rats and guinea pigs to the same particular diet.

Several methods of assaying vitamin C have been proposed. Ordinarily comments upon these should be confined to a communication primarily on methods, except for the fact that a misconception exists in the minds of some as to the accuracy and the full meaning of an assay. At present the methods are at best "crude" (4), and the information obtained is of relative value only. The fundamental fact underlying this situation is that it is impossible to evaluate exactly degrees of effect, as one would determine them by weight or by titration. The best that can be done in a physiological assay is to manipulate dosages until a fairly general and easily recognizable effect is obtained (20). Moreover, animals may vary considerably in initial vigor as well as in response, even when care is taken to obtain as much equality as possible. Furthermore, when one considers that the line of distinction between the state of health and the state of disease, as well as that attribute known as "physiological reserve" (which comes into play in time of stress), are still fruitful subjects for debate, we would expect that the scurvy assay would necessarily be one of the topics involved.

The designation of a method of assay as the "minimum protective dose" method implies that we have reached the stage where we readily recognize complete protection. This is not so, because of our inability to distinguish between the real protection, involving the optimum dose, and apparent protection, which merely is the stage of imminent or incipient scurvy. It has been reported that 15 cc. of milk protected one out of three animals, 30 cc. protected two out of three, and 50 cc. protected all (10). By common consent any one of these doses could be taken to mean the "minimum protective dose" according to whether we mean protecting just a few, about 50 per cent, or practically all animals. Three cc. of fresh canned tomato juice is said to be the "minimum protective dose," but



all animals fed this dose, and so reported, had mild or very mild scurvy at some stage (21, 22). The terms as used experimentally, therefore, may have different meanings and these possible differences in the points of view should be considered.

The short and so-called "minimum curative dose" method which is used for tests on fresh fruits, etc., in season, has the same objections. It implies to the casual observer, who is not familiar with scurvy experimentation, that we can produce standard and exact degrees of scurvy, and that complete curing is possible. Both assumptions are far from proven. Although the method is useful we should appreciate the relative meaning of the results.

Sherman and his pupils (21, 22, 23) have elaborated a numerical scoring system for the statistical treatment of pathological findings. Interpolations (23) have been suggested as well as extrapolations (12). We have also used this system merely as a shorthand method of recording within limits the degree (1, 2 or 3, or mild, moderate, or severe) of the lesion found in various locations. Undoubtedly some system which will summarize pathological findings in a satisfactory form is much needed. We have taken the average of the individual scores of our animals in the same groups, but for the present would prefer to limit the interpretation from the scoring of pathological lesions and their statistical treatment, to only those degrees of difference which we might be able to bring out by the more laborious method of considering each group of individuals separately, and by their general distinction from individuals in other groups.

Another factor enters, namely, in the indirect or "secondary" effects of the vitamins. In ophthalmia, due to lack of vitamin A, infection undoubtedly plays the dominant rôle in the outward symptom. It has recently been suggested that the outward manifestations of beriberi (24) are due to an infection, which is made possible by lack of the antineuritic vitamin. There have been numerous theories as to the infectious nature of scurvy (4). There is no doubt that scorbutic animals are infected. So long as we rely on morbid states and conditions as revealed clinically and pathologically, it does not seem that we will avoid entirely assaying the chances of an animal becoming infected and acting as a host, rather than the primary effect of an absence of vitamin on the animal's own functions. This situation illustrates the more sound logic in using the capacity for growth or other physiologic attributes for assaying vitamins. The lamentable situation, however, is that growth curves in scurvy experiments have not generally been found to be suitable for use, probably on account of the genetic heterozygosity in ordinary stock animals, as well

as latent and chronic disease in commercial guinea pigs. The alien articles of diet, such as milk, which we attempt to test upon this species of animals, together with unnatural methods of administration, also influence the well being of many animals. Undoubtedly progress in the assay of vitamin C may be expected as soon as these mitigating circumstances are improved or eliminated.

All investigators working within the last decade meet on common ground in that they have, if possible, employed growth curves, food intake data, clinical appearances and gross post-mortem findings. Histological findings might help bridge the width of the gap between "minimum protective" and optimum dosages, but would probably not result in any greater sharpness of the decision. For the present the greatest hope of security is to be found in collection of the maximum amount of data possible and the test of conclusions in as many ways as possible. The statistical manner in which investigators often have to treat their data and the dynamic way in which growth, appetite, symptoms, and pathological lesions progress and regress, suggest that the investigator will eventually have to readjust himself and come to analyze nature's manifestations entirely from his own point of view.

#### EXPERIMENTAL PROCEDURE

Commercial guinea pigs were used. These were obtained from the minimum number of breeders possible. A short preliminary period in the laboratory permitted an adjustment to cage life and the elimination of poor eaters, as well as poor consumers of milk. Animals were kept in tinned iron, woven wire cages having 2-1/2 meshes to the inch. Trays or pans, as well as the false bottom screen, were of tinned (dipped) iron. The cages were completely demountable. Ammoniacal vapors attacked these cages at "pinholes" in the tin plating, but they were entirely resistant to alkaline cresol disinfectant. Opal ointment jars of four ounce capacity served as feeding cups, and were wired into a corner of the cage. Water was given by an inverted bottle with a glass tube which projected into the inside of the cage. Animals were weighed, food changed, and water bottles, trays and screens, sterilized every third day. These duties, as well as the feeding of milk, were carried out on Sundays and holidays as regularly as on week days.

*Diet.* We used the diet of Kenny (23) and MacLeod (12), with allowances for differences in the size of the doses of liquid milk fed by varying the butter fat and the skim milk powder contents. The value of the assumption that the amount of milk solids would thereby become equal

for all animals was not completely realized, as the appetite was usually disproportionately restricted in the animals receiving the larger doses of liquid milk. The composition of the diet and dosages of milk are given in Table I.

TABLE I  
COMPOSITION OF DIETS IN TERMS OF ESTIMATED AMOUNTS OF ONE DAY'S RATION

Dose of Milk fed }	62.50 cc.	50 cc.	40 cc.	32 cc.	25 cc.	20 cc.	0. cc. <sup>1</sup>
	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Heated skim milk powder	0.0	1.124	2.1	2.88	3.6	4.15	6.0
Bran	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Rolled oats	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Butter fat	0.0	0.376	0.7	0.92	1.2	1.35	2.0
Sodium chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total dry diet	12.0	13.5	14.8	15.8	16.8	17.5	20
Protein in dry diet	1.88	2.27	2.68	2.97	3.24	3.45	4.15
Total crude protein per day <sup>2</sup>	4.05	4.01	4.07	4.08	4.11	4.14	4.18

<sup>1</sup> Full basal diet, (See Tables II and IV).

<sup>2</sup> Assuming that the ration herein calculated was completely eaten and including the protein in liquid milk fed.

Our first experience during a preliminary experiment with heated skim milk powder revealed that the described method for its devitaminization was not adequate for our particular sample of material. We next made arrangements with Mr. R.S. Fleming of the Merrell-Soule Company, Syracuse, New York, to heat a large batch of powder in an industrial roaster at 110° C for four hours, with constant stirring and continuous aeration, believing that this would be conducive to securing a more certainly devitaminized product, on account of continuous mixing and good exposure to air.

A series of 10 negative control experiments was run on this batch of heated skim milk powder before the regular experiment was started. (See Table II). Since the average was one day less than that in the series of LaMer which we have summarized in Table II, and since two of our contemporaries who are fully familiar with this heated skim milk powder diet told us that occasionally a negative control animal may live longer, than the 34 day period, on a diet completely free from vitamin C, we assumed that the diet was free from vitamin C. Later we became sus-

picious of the sufficiency of the absolute value of this negative control test, and ran another series of seven tests which also are shown in Table II.

We also made a more careful and critical review of the literature and found that as regards scurvy-producing diets, investigators were grouped into two camps, one claiming that their particular diet was free and the other realizing the inadequacy of criteria for judging the test and being satisfied with a diet which would give evidence of producing acute scurvy and death in relatively short experimental periods. The evidence is entirely in favor of the latter group because, as far as any proof is concerned, there is none which shows how long guinea pigs should live; as no diet had been proved to be free from vitamin C, nor has the influence of previous diet upon longevity been studied. Reference to Table II will reveal that the time of heating skim milk powder has been increased from 2 to 4 hours by succeeding observers with the result that longevity has decreased. We do not interpret this as any evidence that the diets used by LaMer (21) and Sherman, LaMer and Campbell (22) or by Kenny (23) contained vitamin C, but rather cite this possible coincidence and conclusion to indicate the impossibility of being absolutely certain one way or another.

As a result of this dilemma, we ran a series of negative controls on a diet of oats, bran, butter fat and salts. This diet, plus tomato juice, we have shown also to be adequate for nutrition. We also ran a series in which 2.2 cc. of the raw milk used in this investigation, diluted to 10 cc. with water, was fed daily for 27 days (being discontinued then on account of causing some aspiration pneumonia in weak and scorbutic animals) with the result that 7 satisfactory experiments lasted 33 to 40 days, or an average of 35.7 days. Since this is 5.5 per cent of the certain minimum protective dose herein reported, we reached the conclusion that there was certainly not more than 2.25 per cent of the minimum protective dose of vitamin C in a day's ration of our full skim milk powder basal diet. Actually this error is still further reduced, as consultation of the Table will reveal that the amount of heated skim milk powder which we fed was from two-thirds of the amount in the full basal diet to no milk powder whatsoever. Consequently this magnitude of error is entirely within the accuracy of the method of assay of antiscorbutic vitamin and need be given no further concern. We have, in presenting these data, gone on the pure assumption, as all others before us, that oats and bran contain no vitamin C.

It should be noted also that our technique differed from that of others using the skim milk powder diet, in that we used one large lot of powder;

TABLE II  
SUMMARY OF NEGATIVE CONTROL EXPERIMENTS

Observer or Source	Number of Experi- ments	Average Initial Weight	Average Maximum Weight	Average Final Weight	Average Loss in Weight	Duration of Experiment		Score	Time of Heating Skim Milk Powder
						Average	Range		
Series I. This investigation	10	gms. 311	gms. 334	gms. 197	per cent 37	days 29.1	days 23-36	16.8	4
Series II. This investigation	7	333	372	218	35	32.1	23-37	18.3	4
Total or Average- Series I and II.	17	320	349	206	36	30.3	23-37	17.3	4
LaMer (21) Sherman LaMer and Campbell (22)}	10	337	372	204	40	30.0	26-34	19.1	2
	15	329	352	202	39				
Kenny (24)	10	353	—	214	39	28.6	21-30	14.9	?
MacLeod (12)	9	—	—	—	—	22.6	19-24	17.1	4

whereas the customary procedure in its preparation is to heat the powder from time to time as needed, thereby placing the burden of experimental credulity upon the method of heating rather than upon the food material itself. The usual custom of testing the diet is to run one to three animals simultaneously, and about three sets during a regular 90-day experiment. Our results would indicate the importance of more numerous tests performed simultaneously, which is another reason for preferring a large, single lot of each dietary constituent.

Two lots of rolled oats, two of bran, and two of butter were used. The first lots were tested in the first series of negative control experiments reported in Table II and the second lots in the second series, respectively. We regretted this procedure but were unable to avoid it as the supply of the first lots of dietary ingredients gave out.

*Quality and Dosages of Milk.* Our preliminary experiments on city delivered certified milk from cows fed on the pasture revealed that 50 cc. were insufficient to protect guinea pigs from scurvy. Believing that this was evidence of destruction through aging, probably as a result of oxidation, we cast about for a means of obtaining milk as soon as possible after milking. We were able to make arrangements with the Hermes-Groves Dairy Company, now a unit of the Liberty Milk Company, for obtaining from their Braeburn Dairy daily at 9 A.M. certified raw milk which had been milked at about 4 A.M. the same morning. The milk was mixed milk obtained from the same three cows for about the first two months of the experiment, but later (owing to three or four accidents, such as fire, reorganization, change in personnel etc.) some changes in animals had to be made. We therefore lost this continuous phase of our experiment, although each cow used throughout our experiment was on 35 pounds of green corn ensilage daily and the regular feeding regimen based upon quantity of milk produced by each cow.

Three groups of experiments were started; raw milk, milk boiled in aluminum, and milk boiled in glass. Dosages of 62.5, 50 and 40 cc. were started in the two boiled milk series and 50, 40 and 32 cc. in the raw series. These dosages stand in relation of 25 per cent increases or 20 per cent decreases. Some replacements were necessary early owing to some acute pneumonia, as well as to some animals, especially in the larger dosage groups, going off the diet. Within six weeks of starting the original experiments, two lower dosage groups had been started in each series, (25 and 20 cc. raw milk, and 32 and 25 cc. dosage groups on boiled milks) owing to the general failure of scurvy to appear in the animals which had been first started.

*Administration of Milk.* Milk was administered by a pipette in preference to feeding in cups, to avoid losses from spilling and to eliminate variations in the willingness of the animal to consume a given amount of milk. This was also done to eliminate variations in the time of consumption which might have affected its vitamin content, especially at room temperature, as well as the consumption of cream layers. MacLeod (12) also fed milk with a pipette. All others have employed cup feeding, at least in part.

It can not be stated that feeding milk by pipette is free from objections. MacLeod found 50 cc. the limit. We accomplished feeding 62.5 cc. per 300 grams body weight but not without considerable effort, a high percentage of animals going off the diet. We feel that both these dosages are too large for a most satisfactory and easily conducted series. The difficulty came apparently from too much swallowed air, the nitrogen probably not being absorbed from the intestine and not being readily propelled along in the larger amounts. Animals on high dosages were occasionally quite "bloated." The condition is probably similar to colic in the nursing infant. Every effort was made to save those responding poorly at the start, but we feel rather that efforts should be confined to starting a sufficient number at the beginning to permit elimination of those which show any tendency to restrict their food intake too much.

The capacity of the stomach of guinea pigs would seem to be at least 25 cc. per 300 gms. body weight, but we have limited the feedings except in special cases to 20 cc. and less. Postmortem examination of animals some time after feeding large doses revealed that functioning of the stomach was grossly perfect. Whey tended to leave rather soon while curds were retained. Hess states that it requires 80 to 100 cc. of milk a day to protect guinea pigs, (weight of animals not stated) and we infer that very few will consume this amount by cup, even when proffered over a long period of time. After all things have been said, we believe that feeding by pipette is nevertheless the more desirable, since it is conducive to the greater accuracy and certainty, but it is expensive and time consuming. In practice it amounts, in either method of feeding, to the obtaining of a sufficient number of satisfactory animals. The rate of feeding milk by hand is about 80 cc. per hour under average conditions.

The milk was measured in 25 cc. graduates. Allowances were made by weight for any losses in feeding by catching that which was slobbered or spat out, on a cloth and by wiping off the chin. After several days most animals learned to drink without loss. The pipettes were of hard glass, of about 7 cc. capacity, and had a narrow, practically cylindrical tip

about one half inch long. A commercial rubber bulb of  $\frac{1}{4}$  ounce capacity was used at the upper end. Care was taken not to trap milk in the rubber bulb. The glassware and rubber bulbs were sterilized each day in alkaline hypochlorite solution, and also after feeding any pneumonic pigs which we may have tried to save. Animals with colds or pneumonia were isolated from the healthy ones and fed separately.

*Boiling and Care of the Milk.* It required an average of 19.3 ( $\pm 2.2$ ) minutes to bring milk to the boiling point in the aluminum sauce pan and 26.9 ( $\pm 4.2$ ) minutes in the glass beakers. (The standard deviations are given in parentheses). The rate was the natural one for the aluminum, but it was pushed in the case of the glass as rapidly as possible, without securing a scorched taste, in order to bring the time for glass as nearly as possible to that for aluminum. Light boiling was continued 5 minutes, which is the time used by Hess (4) and also given by Brennemann (3). Some pediatricians use less time. Three quarts of milk were boiled at one time, which represents more than a probable maximum in the home or hospital.

As soon as practicable the hot milk was poured through a cheese cloth in a glass funnel into pint bottles and stored in an electric refrigerator kept below the freezing temperature. No milk froze up to the time of the last feeding. The milk was at room temperature when fed, being taken as needed out of a pint bottle, exposed to the air but covered with a cap. Equalization of any errors introduced at this stage occurred, since the order of feeding differed from day to day. The utensils were thoroughly washed daily, the aluminum pan being polished with steel wool. The pint milk bottles were sterilized daily in the oven of a gas range. The age of the milk fed, considering time in transit and averaging early and late feeding times, was practically always under 10 to 12 hours.

#### DISCUSSION OF EXPERIMENTAL RESULTS

There are given in Table III the analytical results from the boiling of water and milk in aluminum. The amounts of aluminum taken up are to be regarded as within the limits of the unavoidable. Such amounts could easily come from sources other than aluminum cooking utensils such as dust contamination as well as hydrolysis of rock and soil on weathering in water sheds and in rivers. There are also given in the table the results of analyses performed upon whole milk powder. Since liquid milk is one-eighth solids, dividing these results by 8 would bring the concentration of aluminum in remade whole milk to 0.4 to 0.5 parts per million. These results are therefore in agreement with those of other milks examined.



TABLE III  
ALUMINUM CONTENT OF WATER AND OF MILK BOILED IN ALUMINUM

Material Tested	Time in Boiling minutes	Duration of	Amount taken		Aluminum		Remarks
			Boiling cc.	One Analysis	Before parts per million	After parts per million	
Tap water	—	1	1000	250	0.6	0.8	From Pittsburgh City Main. Pan #19, 2-S Metal.
Certified milk	19	5	3000	200	0.5	0.6	Fresh, 6 hours old. Pan #19, 2-S Metal.
Store milk	60	30	— <sup>a</sup>	250	0.6	1.0	Age of milk unknown. Stirred with aluminum spoon inter- mittently. Pan #1, 3-S Metal.
Commercial whole milk powder	—	—	—	—	3.2	—	
Lot A	—	—	—	—	3.0	—	
" B	—	—	—	—	4.0	—	
" C	—	—	—	—	4.0 <sup>b</sup>	—	
" D	—	—	—	—	—	—	

<sup>a</sup> More than 1000 cc.

<sup>b</sup> Preliminary determination.

The analyses were performed colorimetrically with the dye aurin tri-carboxylic acid. Iron was separated by the cupferron method. Full recoveries of added aluminum were obtained. The expression of the results is in round numbers on account of an experimental error of about 10 per cent, as well as the concentration of aluminum being the least that is susceptible to handling conveniently.

There is given in Table IV a summary of the animals receiving the full basal diet plus 15 grams of fresh spinach daily. These animals were fed for 89 days or more, and were for the purpose of furnishing control autopsy material. They were not for the purpose of positive controls in the true sense, since these latter experimental animals are properly found at the top of a scurvy series. Controls on the basal diet plus 15 grams of spinach daily should, however, be identical with those which one would obtain at the top of an experimental series, if several conditions did not enter. We do not know at present that the skim milk powder diet is optimum in all respects. It is known, but not well known, that animals receiving protective doses of vitamin C from different sources, but the same basal diet, may differ in growth<sup>1</sup>.

The growth of these animals was satisfactory, because others were eliminated early that failed to react so. Since there were fewer animals than the number of days on which animals of the regular experiment were killed for autopsy, we dispensed with the service of an autopsy standard on several days, but held animals in readiness for use if needed. The condition of these autopsy controls was good except that one gave evidence of having had a respiratory impediment, probably pneumonia, as judged by rotated and somewhat angulated ribs. Broadening of the ribs, especially the junctions, was found in two; but the relations of the finer details were absolutely normal to the naked eye. Our early suspicion that broadening of the ribs was of scorbutic origin, possibly being a reaction to less than the optimum amounts of antiscorbutic agent, was neither confirmed or disproven. The condition was not infrequently found in animals concerning which no suspicion of scurvy was ever had.

Since we used commercial animals, we realized the necessity of controls for estimating their quality. Twenty-six animals became available early in the preliminary period in which the response to milk was being studied. These were killed and autopsied and found to be free of scurvy. Later four more were obtained, remaining from a group of substitutes, and were likewise free of scurvy. These animals, however, showed some pneumonic conditions which we attributed in part to accidents during the preliminary

<sup>1</sup> Personal communication from Prof. W. H. Eddy.

TABLE IV  
SUMMARY OF RECORDS OF ANIMALS FOR AUTOPSY CONTROL

Animal Number	Original Weight gm.	Final Weight gm.	Gain gm.	Days Duration of Experiments	Gain per Day gm.	Total Food Eaten gm.	Food per day gm.	Gain per gm. Food Eaten gm.	Note on Pathological Examination
257	323	576	253	113	2.24	2073	18.34	.122	No scurvy
312	348	573	225	90	2.5	1909	21.21	.118	No scurvy
304	342	733	391	90	4.33	2130	23.67	.185	No scurvy
327	295	575	280	89	3.15	1753	19.70	.160	No scurvy
334	278	450	172	117	1.47	1963	16.77	.087	No scurvy
395	325	658	333	102	3.26	1986	19.47	.168	No scurvy
404	314	700	386	112	3.45	2504	22.36	.154	No scurvy
407	328	567	239	112	2.13	2203	19.67	.108	No scurvy
421	318	726	408	113	3.61	2805	24.82	.145	No scurvy certain.
									Nervous pig throughout experiment.
571	373	746	373	89	4.19	2360	26.52	.158	No scurvy
576	373	578	205	90	2.28	1805	20.06	.114	No scurvy
Average	329	626	297	102	2.96	2135	21.14	.138	

period. There was also some chronic lung disease more or less of which is always found in commercial animals. Later 50 animals came to autopsy, which included members from all lots of animals which we purchased; two of these animals were regarded as doubtful but given zero scurvy scores, and two were given scurvy scores of "1" each (maximum score = 27).

For all animals the chances are then two in seventy, or about 3 per cent, of meeting traces of scurvy existing previous to entering the laboratory. We would call attention to the general absence of any control of this sort having been regularly exercised by investigators, especially those using commercial animals, and would certainly feel that this source of error should be carefully controlled in the future. Cavanaugh, Dutcher, and Hall (8) have called attention to the necessity of examining animals for signs and symptoms of scurvy before use. All investigators attempt a short preliminary period of feeding before starting the regular experiment. It is questionable, however, whether even these precautions will suffice, particularly if we employ histological evidence, as the lesions of scurvy have not been proved to heal by resolution or without leaving marks of their previous existence (25). It is probable that the most satisfactory solution of this matter will come in breeding animals in the laboratory after the methods of scurvy experimentation become sufficiently facile to permit the employment of smaller numbers than are at present required.

A summary of the clinical and pathological scorbutic findings is given in Tables V, VI and VII. The data obtained from animals surviving the full experimental period are given separately from those obtained from animals which died previous to the expiration of 90 days, but after the 45th day. The experiment passed through the vicissitudes of an acute pneumonia epidemic at the time of the general human epidemic of December, 1928. By reference to Tables VIII, IX, and X, those animals showing no pathological lesions whatsoever, other than those of scurvy, or simulating scurvy, can be identified, so one is privileged to make a comparison on the basis of only the entirely healthy animals if desired.

The presentation of the clinical data is self explanatory, with the exception of the doubtful column. This column lists those cases in which we deemed that the animal would bear watching but on which we were unable to make a positive diagnosis. Every investigator discovers this dilemma because all scorbutic animals become "nervous" or "touchy" while all nervous ones may not have scurvy. The clinical information as listed refers to a determination of the worst condition which was reached at any time during the course of an experiment. Those with no scurvy were obviously free the entire period. The tables do not enumerate re-

lapses or remissions. Although for the most part the scorbutic state is dynamic, some stationary cases were observed.

The method and value of scoring has been previously mentioned. Since a record of pathological findings is distinct from a pathological diagnosis, we have recorded rigorously those conditions which were present even though we were of the opinion that they were not always primarily and in strict fairness to be attributed to the experimental treatment. The latter is indicated in the column under "remarks." Old hemorrhages present quite a problem, as hemosiderin may remain for a long time *in situ* and confuse the unwary, though due to pre-experimental circumstances. Also the post-mortem scores may be exaggerated in some cases, owing to the fact that the hemorrhage *per rhexis* is of traumatic origin (24), while the tendency to have a hemorrhage, is undoubtedly scorbutic. We have ample evidence from other experience that an animal on the scorbutic threshold, presumably with no lesions present which the naked eye would discern, can have a severe muscular hemorrhage as a result of exertion, and give a higher score than his so-called peers or equals. We have used ether in terminating experiments, and thereby avoided the possibility, encountered by some others, of chloroform producing hemorrhages.

The peculiarities of this series of experiments are found chiefly in the experiments in which the larger doses of milk were fed to animals not surviving the full experimental period. One animal receiving 50 cc. of raw milk had definitely scorbutic ribs. One animal receiving 62.5 cc. milk boiled in aluminum had an attack of respiratory disease and pulmonary abscesses containing greenish pus. We suspected arthritis, even ante-mortem, and the wrists were acutely swollen, being much larger than any scorbutic wrists which we had seen up to that time. There was no hemorrhage or congestion about these joints upon gross post-mortem examination and no evidence of scurvy was found elsewhere. Two animals in the 50 cc. group had traces of old shin hemorrhages which we believe were acquired before or perhaps very early in the experiment as result of pre-experimental circumstances. Likewise two animals, one receiving 62.5 cc. of milk boiled in glass and another 50 cc., presented conditions which we assigned to other than direct experimental causes. The effect of these, if one is to interpret them for the worst, is to push the certain protective dose higher into dosages which it is not only impossible but impractical to feed. In view of what we have said, as well as the result of our examination of the quality of commercial animals which we used, and the high incidence of alimentary disturbances in animals in the high dosage groups,

we have disregarded these occasional irregularities, presenting them here only for the sake of completeness.

There are, nevertheless, enough data to allow us to draw conclusions after the complete rejection of all animals dying during the course of the experiment, in which case the number of unusual results is considerably decreased. The general trends take precedence over too weighty an influence of one experiment on account of the paucity of data in individual groups (even though the total animals used are large in number). A tendency exhibited by, or conclusion drawn from, one group is moreover expected to be strengthened by that from other groups.

Twenty cubic centimeters of raw milk and 25 cc. of each of the boiled milks are practically on a par and 40 cc. of the raw milk and 50 cc. of each of the boiled milks are also about on a par. In the former case practically all animals were afflicted with certain scurvy, either diagnosed by clinical observation or by post-mortem examination or both, whereas in the latter there was a general freedom from scurvy both clinically and pathologically. Intermediate groups bring out intermediate effects. These data, therefore, support the conclusion that boiling of milk, as in our experiments, reduced the concentration of the antiscorbutic potency to 80 per cent of its original value, and that there has accordingly been a 20 per cent loss. They also show that there was no detectable difference between boiling in the two types of vessels. Since glass may be taken for the present as an example of an inert standard, it follows that aluminum is equally good, and that the destruction of that amount of vitamin which did occur, is referable to the boiling process alone.

These data conform to the existence of a range of apparent protective doses, in contradistinction to a precise and one-level protective dose, unless such level be an arbitrary or a statistical one. This has been the experience of other investigators(10). It is also the point of view of Hess (4) when he says that it takes a pint of milk a day to protect an infant, in that he refers to the elimination of practically all cases and not to an incidence of 50 per cent. Moreover, such an experience is well known in pharmacological and toxicological studies, (26), and it would seem important for future investigators to bear this in mind in formulating their schemes of attack when assaying vitamin C.

The individual gains in weight and food intake are given in Tables VIII, IX, and X. No animals are included which showed any pathology other than scurvy, even though the pulmonary lesions were entirely localized or of an incipient type. The reason for this exclusion is that we have found by extensive study that this condition is frequently asso-

TABLE V  
SUMMARY OF CLINICAL HISTORIES AND POST-MORTEM EXAMINATION OF GUINEA PIGS  
FED RAW MILK

Group Dose	Dura- tion	Guinea Pig No.	Degree of Clinical Scurvy				Autopsy		
			Free	Doubt- ful	Slight	Mod- erate	Severe	Score	Remarks
cc. 50	days								
	90	233	x					0	Ribs over grown. Cause?
	90	236	x					0	
	90	285	x					0	
	90	358		x				0	
	90	420		x				0	
	90	459	x					2	
	Av. 90 days		6		0	0	0	0.33	
	66	243	x					0	
	Av. less than 90 days		1		0	0	0		
Grand Total		7		0	0	0	0.33		
40	90	223	x					0	
	90	227	x					0	
	90	231	x					0	
	90	247	x					0	
	90	269	x					0	
	90	230	x					0	
	90	385	x					0	
	Av. 90 day and Grand Total		7		0	0	0	0	
32	90	249		x				2	
	90	263				x		2	
	90	282	x					0	
	90	284		x				1	
	90	306	x					0	
	90	402		x				0	
	90	504			x			0?	
	Av. 90 day		5		1	1		0.7	
	80	328		x				1.5	
	85	311			x			1.5	
	Average less than 90 days		1		1			1.5	
	Grand Total		6		2	1	0	0.9	

TABLE V—continued

25			Degree of Clinical Scurvy					{ Recent haem. violent injury.
90	391	x				2		
90	396			x		1		
90	416		x			1.5		
90	445		x			0.5		
90	450	x				3.		
90	452	x				1.5		
Av. 90 day and Grand Total			5	1	1	1.6		
20	90	509		x			5	
	90	511				x	1	
	90	514					x	7
	90	517					x	6
	90	523				x		6
	90	525		x				0?
	90	527				x		3.5
	Av. 90 days			2	1	2	2	4.1
	80	512				x		11.5
	87	515				x		3
	Average less than 90 days			3	2	0	0	7.25
	Grand Total			3	2	2	2	4.8

ciated with poor growth and appetite and with the shortening of life of animals receiving no vitamin C in their diets (negative controls). Although in some animals this does not appear to be the case, we have, nevertheless, adopted the policy of eliminating such experiments from immediate consideration.

By comparison with those animals recorded in Table II it is seen that the animals fed milk by pipette grew rather poorly. We are inclined to attribute this to lack of appetite in some cases, since the growth per gram of food consumed was sometimes as great as in those animals fed the basal control. It should be noted, however, that these latter animals received spinach which furnished additional vitamins, whereas the experimental animals received only milk. We do not know what the effect of heating the milk powder used in our diet was on other or unknown vitamins. As the data stand, there are insufficient experiments in each group to strike a significant average growth, considering the possible individual variation which is within normal limits (see Nos. 334 and 421, Table II).



**TABLE VI**  
**SUMMARY OF CLINICAL HISTORIES AND POST-MORTEM EXAMINATION OF ALL GUINEA PIGS FED MILK BOILED IN ALUMINUM**

Group Dose	Dura- tion	Guinea Pig No.	Degree of Clinical Scurvy				Autopsy		
			Free	Doubt- ful	Slight	Mode- rate	Severe	Score	Remarks
cc. 62.5	days								
	90	235	x					0	{ Died suddenly. Lung Abscess. No certain scurvy macroscopically. Foci of infection in wrist found histologically.
	90	303	x					0	
	Av. 90 days		2	0	0	0	0		
	81	360	No diagnosis made		arthritis sus- pected		3?		
	63	273	x				0		
	54	248	x				0		
	63	364	x				0		
	Av. less than 90 days		4	0	0	0	0.75		
	Grand Total		6	0	0	0	0.5		
50	90	220	x					0	
	90	262		x				1	
	90	276	x					0	
	90	382	x					0	
	90	390	x					0	
	90	403	x					0	
	90	408		x				0	
	90	473	x					0.5	
	Av. 90 days and Grand Total		8	0	0	0	0.19		
	40	90	255		x				0
90		272	x					0	
90		325	x					0	
90		330		x				0	
90		424		x				3	
90		469		x				1	
Av. 90 days		6	0	0	0	0.67			
68		302	x					0	
Av. less than 90 days		1	0	0	0	0			
Grand Total		7	0	0	0	0.6			

TABLE VI—continued

			Degree of Clinical Scurvy		
			x		
32	90	386			0?
	90	518	x		0
	90	567		x	6
	90	570		x	0
	Av. 90 days		1	2	1.5
	78	412	x		1
	57	569		x	1
	Av. less than 90 days		1	1	1
	Grand Total		2	3	1.3
25	90	524		x	7
	90	532		x	3
	90	533	x		1
	90	554		x	1
	90	568		x	1
	Av. 90 days		1	3	2.6
	51	520		x	0
	81	522		x	7
	48	530		x	0.7
	Av. less than 90 days		0	2	2.3
	Grand Total		1	5	2.5

Easy bleeder

Cavanaugh, Dutcher and Hall (8) used growth curves, but their data are more numerous per group than ours. Kenny (23) used growth curves in tomato juice experiments. MacLeod(12) omitted consideration of growth in milk experiments similar to ours.

The data obtained from the experiments in which 25 cc. and 32 cc. of milk boiled in glass were fed are unique in that they show these animals tended to exceed some other animals in growth or were about as good as the best. However, there is here shown a tendency for a greater degree of scurvy to appear at autopsy. This might be expected under these circumstances, and definitely indicates that more data than we have are necessary before relying upon growth curves.

TABLE VII

SUMMARY OF CLINICAL HISTORIES AND POST-MORTEM EXAMINATION OF ALL GUINEA PIGS  
FED MILK BOILED IN GLASS

			Degree of Clinical Scurvy				Autopsy		
Group Dose	Dura- tion	Guinea Pig #	Free	Doubt- ful	Slight	Mode- rate	Severe	Score	Remarks
cc. 62.5	days								{ Prob. not an ex- perimental affair.
	90	335	x					0	
	90	480	x					1/2?	
	90	483	x					0	
	Av. 90 days		3	0	0	0	0	0.16	
	52	349	x						
	Av. less than 90 days		1	0	0	0	0	0	
	Grand Total		4		0	0	0	0.1	
50	90	250	x					0	{ May partially or all have been pre-experimental
	90	264			x			0	
	90	305	x					0	
	90	345	x					0	
	90	427	x					0	
	Av. 90 days		5		0	0	0	0	
	81	455	x					0	
	45	379	x					3?	
	52	359	x					0	
	45	381	x					0	
	Av. less than 90 days		4		0	0	0	0.75	
	Grand Total		9		0	0	0	0.33	
40	90	281	x					0	
	90	291			x			2	
	90	371	x					0	
	90	388			x			1?	
	Av. 90 days		3		1	0	0	.75	

TABLE VII—continued

			Degree of Clinical Scurvy				
40	53	324		x			2
	66	369		x			2
	51	368	x				0
	70	307	x				0
	Av. less than 90 days		3	1	0	0	1
	Grand Total		6	2	0	0	0.9
32	90	439			x		5.5
	90	400			x		5.0
	90	475		x			1.0
	90	498		x			0.0
	90	537			x		1.5
	Av. 90 days		2	1	2	0	2.6
	66	497			x		1
	53	573		x			0
	52	448			x		5.5
	Av. less than 90 days		1	1	1	0	2.2
	Grand Total		3	2	3	0	2.4
25	90	543		x			1
	90	544		x			6.6
	90	550	x				3.5
	90	558	x				1
	Av. 90 days		4				3
	72	564			x		10
	60	542		x			0?
	Av. less than 90 days		1				5
	Grand Total		5	0	1	0	3.7

Recent Haemorrhage.

In the last column of Tables VIII, IX, and X, the clinical condition of each animal is entered. No pathological condition other than scurvy was to be found in the experiments recorded in these tables. These data lead to the same conclusion that we drew from consideration of all animals, (Tables V, VI, and VII) namely, that about 20 per cent of the vitamin C was destroyed in boiling.

TABLE VIII  
90 DAY EXPERIMENT ON RAW MILK

Group Dose	Animal No.	Weight		Gain	Food Consumed			Gain per gm. Food	Scurvy Score	Clinical <sup>1</sup> Status
		Start	End		Dry Food	Milk (dry basis)	Sum			
cc. 50		gms.	gms.	gms.	gms.	gms.	gms.			
	420	317	467	150	826	596	1422	.105	0	Doubtful
	236	304	456	152	967	568	1535	.099	0	0
40	230	338	523	185	1257	506	1763	.105	0	0
	247	292	494	202	1109	439	1548	.130	0	0
	269	364	630	266	1373	586	1959	.136	0	0
	231	315	480	165	959	473	1432	.115	0	0
32	282	334	481	147	1120	399	1519	.097	0	0
	306	371	468	97	1096	444	1540	.063	0	0
	504	307	500	193	1316	371	1687	.114	0	0
	284	388	412	24	815	467	1282	.019	1	Doubtful
25	391	331	491	160	1460	309	1769	.090	2	0
	396	344	378	34	1016	321	1337	.025	1	+
	416	297	476	179	1189	281	1470	.122	1.5	Doubtful
	445	283	452	169	1175	264	1439	.118	0.5	Doubtful
	450	318	478	160	1148	273	1421	.113	3	0
	452	293	450	157	1167	276	1443	.109	1.5	0
20	509	306	408	102	1194	231	1425	.072	4	Doubtful
	511	307	485	178	1624	231	1855	.096	1	++
	514	324	272	-52	1169	242	1411	—	7	+++
	517	322	434	112	1536	242	1778	.063	5	+++
	523	345	473	128	1616	259	1875	.068	4	++
	525	321	396	75	1082	242	1324	.057	0	Doubtful

<sup>1</sup> "Doubtful" means not certainly diagnosable, and plus marks the degree of scurvy.

### SUMMARY AND GENERAL DISCUSSION

An experiment has been reported on the destruction of Vitamin C in milk by boiling, based upon the development of experimental scurvy in guinea pigs. The data do not permit the mathematical expression of results with a precision of a few per cent. They do, however, indicate that there was no detectable difference in the destruction of vitamin C between boiling milk in aluminum and boiling in glass. The loss of vitamin is placed at about 20 per cent. If glass may be taken as an inert standard, it is certain that no detectable catalysis by the aluminum is evident; rather, as was to be expected, the effect is referable to the cooking *per se*.

The data are also of interest in that our winter milk was approximately if not actually of the same potency as the best summer milk obtained from cows on pasture(9). If a pint of ordinary city milk is required to insure the protection of infants from scurvy, and if such milk protects guinea pigs when fed at the 80 to 100 cc. level (4), it can be said that the milk used by us was at least twice as potent, even in the boiled state, as ordinary city delivered milk in New York City. We would estimate that

TABLE IX  
90 DAY EXPERIMENT ON MILK BOILED IN ALUMINUM

Group Dose	Animal No.	Weight		Gain	Food Consumed			Gain per gm. Food	Scurvy Score	Clinical <sup>1</sup> Status
		Start	End		Dry Food	Milk (dry basis)	Sum			
cc.		gms.	gms.	gms.	gms.	gms.	gms.			
62.5	235	296	553	257	799	698	1497	.172	0	0
50	276	290	441	151	839	546	1385	.109	0	0
	382	357	517	160	996	669	1665	.096	0	0
	473	306	414	108	954	574	1528	.071	0.5	0
	390	313	458	145	869	585	1454	.100	0	0
40	255	345	561	216	1317	518	1835	.118	0	Doubtful
	325	347	525	178	1040	523	1563	.114	0	0
	469	290	440	150	820	438	1259	.119	1	Doubtful
	272	323	441	118	1004	484	1488	.079	0	0
32	570	316	405	149	973	377	1350	.110	0	+
	518	364	554	190	1240	439	1679	.113	0	+
25	554	329	390	61	942	309	1251	.048	1	++
	568	344	449	105	1094	321	1415	.074	1	+
	533	387	518	131	1306	360	1666	.079	1	Doubtful

<sup>1</sup> "Doubtful" means not certainly diagnosable, and plus marks the degree of scurvy.

200 cc. daily of our raw milk less than 12 hours old would probably suffice for the infant, but of course we would recommend more for the sake of safety. Dutcher(27) has had the general experience that 30 cc. of milk will protect 250 gram guinea pigs, and his observation is to be accounted for by the fact that poorly produced and handled milk is an unknown quantity at Agricultural Experiment Stations and Colleges in the United States.

The data are of importance in that the destruction was of the order of only 20 per cent, which is in complete conformity with the historical

fact that boiled milk has adequately served in the past as the sole or chief source of antiscorbutic vitamin for some artificially fed infants. However, this historical fact applies to less strenuous circumstances than operate in a modern city; and therefore, by elimination, the problem is to obtain potent milk to begin with.

The data are also of considerable interest in emphasizing the necessity of some rigorous definition of the term "protective dose" at least for the

TABLE X  
90 DAY EXPERIMENT ON MILK BOILED IN GLASS

Group Dose	Animal No.	Weight		Gain	Food Consumed			Gain per gm. Food	Scurvy Score	Clinical <sup>1</sup> Status
		Start	End		Dry Food	Milk (dry basis)	Sum			
cc. 62.5		gms.	gms.	gms.	gms.	gms.	gms.			
	335	288	470	182	540	675	1215	.150	0	0
	480	316	516	200	631	743	1374	.145	1/2?	0
	483	292	499	207	708	687	1395	.148	0	0
50	250	285	480	195	962	534	1496	.130	0	0
	305	326	527	201	874	613	1487	.135	0	0
	427	344	552	208	1152	647	1799	.116	0	0
40	291	274	504	230	1227	411	1638	.140	2	Doubtful
32	439	265	488	223	1493	321	1814	.123	4.5	++
	400	335	493	158	1490	399	1889	.084	5.0	++
	475	297	500	203	1311	354	1665	.122	1.5	0
	537	400	665	265	1354	478	1832	.145	1.5	Doubtful
25	544	367	634	267	1451	343	1894	.141	6.5	Doubtful
	550	337	659	222	1410	315	1726	.129	3.5	0

<sup>1</sup> "Doubtful" means not certainly diagnosable, and plus marks the degree of scurvy.

basis of assaying, even if not for computing optimum allowances for the animal. The data fall in line with the experience of pharmacologists who recognize a latitude between the least dose producing a given degree of an effect and a dose producing it in practically all cases(26). This therefore brings into an assay of this type a statistical aspect, regardless of whether arithmetical computations employed by statisticians are or are not performed. It might be imagined that discrepancies between the value of equal-size doses would occur in an animal from day to day, much as with single doses of a medicament given to different animals at the same time. It would appear plausible under these circumstances that

unequal effects from equal doses of vitamin repeated daily would reach an average in the long run, but the theory unfortunately does not work out in practice, as evidenced by remissions of disease in the same animal, or by a partial incidence of disease in animals at a single dose level. When this is realized, it is only one step further to conclude that the agreement in a small amount of data is certainly not indicative of high accuracy, even in assays involving repeated daily feeding.

The growth curves and food intake data were of no help in drawing our conclusions. This is not to be interpreted as a general prediction for the future.

Potent milk is absolutely essential to the success of an experiment of this sort. While the pipette feeding of milk to guinea pigs does not appear to be the best biological practice, and apparently influences appetite adversely, we believe that it is superior to cup feeding, which introduces spillage, aging, cream consumption, and varying daily doses.

#### CONCLUSIONS

The amount of antiscorbutic vitamin destroyed by lightly boiling three quarts of milk for 5 minutes in a glass beaker or in an aluminum stew pan was, within the limits of accuracy of experiments of this kind, found to be approximately 20 per cent.

The quality of the winter milk used and obtained from ensilage-fed cows was better than any heretofore reported upon and almost, if not actually, as potent as the best summer milk obtained from cows on pasturage. This points to a forgotten but possible high potential value of milk as a carrier of vitamin C.

The amount of aluminum contributed to Pittsburgh tap water and fresh milk upon boiling in an aluminum vessel is practically nil, being 0.1 to 0.4 parts per million.

#### REFERENCES

1. Sherman, H. C., *Chemistry of Food and Nutrition*, 3rd ed., New York, 1926.
2. Brennemann, J., *Jour. Amer. Med. Assoc.*, 1916, LXVII, 1413.
3. Brennemann, J., See Abt., I. A., Editor, *Pediatrics* by Various Authors. Vol. II, Philadelphia, 1926.
4. Hess, Alfred F., *Scurvy, Past and Present*. Philadelphia and London, 1920. With complete early Bibliography.
5. Hess, A. F., and Fish, M., *Amer. Jour. Dis. Child.*, 1914, VIII, 386.
6. Johnson, T. L., and Norton, J. F., *Food and Health Education*, 1927, V, 89.
7. Hart, E. B., Steenbock, H., and Smith, D. W., *Jour. Biol. Chem.*, 1919, XXXVIII, 305.
8. Cavanaugh, G. W., Dutcher, H. A., and Hall, J. S., *Jour. Indust. and Eng. Chem.*, 1924, XVI, 1070.
9. Dutcher, R. A., Eckles, C. H., Dahle, C. D., Mead, S. W., and Schaefer, O. G., *Jour. Biol. Chem.*, 1920, XLV, 119.



10. Hart, E. B., Steenbock, H., and Ellis, N. R., *Jour. Biol. Chem.*, 1920, XLII, 383.
11. Hess, A. F., Unger, L. J., and Supplee, G. C., *Jour. Biol. Chem.*, 1920, XLV, 229.
12. MacLeod, Florence L., *Jour. Amer. Med. Assoc.*, 1927, LXXXVIII, 1947.
13. Hess, A. F., *Jour. of Indust. and Eng. Chem.*, 1921, XIII, 1115.
14. Rice, F. E., and Miscal, J., *Jour. Dairy Sc.*, 1923, VI, 261.
15. McCollum, E. V., Simmons, N., and Becker, J. E., *Proc. Soc. Exptl. Biol. and Med.*, 1927, XXIV, 953.
16. Rideal, E. K., and Taylor, H. S., *Catalysis in Theory and Practice*, London, 1926.
17. Mellor, J. W., *Inorganic and Theoretical Chemistry*, Vol. V, London, 1924.
18. Waters, C. E., *Jour. Indust. and Eng. Chem.*, 1921, XIII, 901.
19. Hunziker, O. F., Cordes, W. A., and Nissen, B. H., *Jour. Dairy Sc.*, 1929, XII, 140.
20. Schwartz, E. W., *Nations Health*, 1927, IX, 13.
21. LaMer, V. K., Dissertation, Columbia University, New York City, 1921.
22. Sherman, H. C., LaMer, V. K., and Campbell, H. L., *Jour. Amer. Chem. Soc.*, 1922, XLIV, 165.
23. Kenny, C. L., Dissertation, Columbia University, New York City, 1926.
24. Matsumura, S., *et al.*, *Jour. Amer. Med. Assoc.*, 1929, XCII, 1325.
25. Meyer, A. W., and McCormick, L. W., *Studies on Scurvy*, Stanford Univ. Pub. Med. Sc., Vol. II, No. 2, Stanford University Press, 1928.
26. Schwartz, E. W., *Toxicity of Barium Carbonate to Rats*, *Bull. U. S. Dept. of Agr.* 915, Washington, D. C., 1920.
27. Dutcher, R. A., *Factors, Influencing the Vitamin Content of Cows Milk*. Penn. State Coll. Agr., 1924.

---

# A STUDY OF THE VITAMIN B COMPLEX\* OF RED KIDNEY BEANS AND POLISHED RICE†

BY J. H. AXTMAYER

(From the Department of Chemistry, University of Porto Rico, Rio Piedras)

Received for Publication—September 10, 1929

IT DOES not seem necessary to review in this paper the literature pertaining to vitamin B and the researches which have shown its multiple nature, since this has been summarized and discussed in the recent literature pertaining to the complex nature of vitamin B, especially in an editorial review by Sherman (1928) and in a dissertation by Chase (1928).

Sherman and Axtmayer (1927) devised a method by means of which they determined which of the two now recognized factors of the vitamin B complex, *viz.*, the antineuritic or vitamin B and the pellagra-preventive or vitamin G, is the limiting factor in whole wheat flour and in milk powder. In view of the fact that highly polished rice and red kidney beans, served either separately or together, is a dish which is consumed daily by the people of Porto Rico, at the sacrifice of other more nutritive foodstuffs, it was decided to apply this method to determine whether vitamin B ( $B_1$ ) or vitamin G ( $B_2$ ) is the limiting factor of the "vitamin B complex" of this mixture. It has been shown that foodstuffs may be richer in one of the factors than in the other, hence a reinvestigation and reinterpretation of the previously recorded "vitamin B values" of foodstuffs becomes a necessity in order to know whether there exists any supplementary relationship between any two insofar as the vitamin B complex content is concerned. The relative richness of any one factor should also be determined because the concentration of these two factors is not the same in all foods, and a foodstuff rich in one may be relatively poor in the other.

## EXPERIMENTAL

The experiments were conducted according to the methods of Sherman and Spohn (1923), and Sherman and Axtmayer (1927) for the quantitative determination of vitamin B values of foods. These methods involve placing healthy young rats when 28 to 29 days old upon a vitamin B-free diet which is not only adequate but approximately optimal (for growth of rats) in all other respects. The basal diet consists of purified casein,

\* The term "vitamin B complex" is used in this paper to signify the combination of the thermo-labile factor, vitamin B ( $B_1$ ) and the thermo-stable factor, vitamin G ( $B_2$ ).

† Published as Contribution No. 1 from the Department of Chemistry, University of Porto Rico, Rio Piedras, P. R.

18; Osborne and Mendel (1919) salt mixture, 4; butterfat, 8; cod-liver oil, 2; corn starch, 68 per cent. The corn starch was such as has been found by Osborne, Wakeman and Ferry (1919) to be free from vitamin B.

The foods tested in the experiments described in this paper were polished rice, autoclaved yeast, and red kidney beans. The rice and beans, which were of the same kinds as used in the Porto Rican homes, were purchased from a local grocer and were freed from dust and extraneous matter. They were then ground separately, the rice in a mortar and the beans in a meat grinder. Dried baker's yeast was placed in uncovered Petri dishes to a depth of 15 mm. and heated in an autoclave at 15 pounds steam pressure for 150 minutes; then, after cooling and drying in the air, it was ground to a powder in a mortar.

These test foods were fed separately or in combination as sole sources of vitamin B to the standard test animals on the basal vitamin B-free diet.

The multiple nature of vitamin B having been established, it was deemed advisable to determine the relative richness of the factors of the vitamin B complex in polished rice and red kidney beans by the same method which is based on the fact that if better growth is induced by the feeding of a mixture of foods as the source of vitamin B complex than is obtained when double the amount of each is fed separately, then, all other conditions having been properly controlled, the conclusion is justified that the better growth is due to a supplementation of one of the factors of the vitamin B complex by the other.

#### NON-SUPPLEMENTARY RELATION BETWEEN POLISHED RICE AND RED KIDNEY BEANS

After some preliminary work a series of experiments was performed in which the experimental animals (rats) received as the sole source of vitamins B and G the following daily (six days per week) portions of the test foods: 0.8 grams of ground red kidney beans; or 0.8 grams of ground polished rice; or 0.8 grams of autoclaved yeast; or a mixture of 0.4 grams each of the rice and red kidney beans; or a mixture of 0.4 grams each of the rice and autoclaved yeast; or a mixture of 0.4 grams each of the beans and autoclaved yeast; with the negative control experiments in which animals received the vitamin B-free basal diet. The 0.8 grams level of feeding the supplements was chosen in order that the results obtained be directly comparable with those obtained by Sherman and Axtmayer (1927).

The average weight curves of those animals receiving daily portions of 0.4 grams each of the red kidney beans and the autoclaved yeast;

0.4 grams each of the polished rice and the red kidney beans; 0.8 grams of the autoclaved yeast alone; 0.8 grams of the red kidney beans alone, and the negative controls, are shown in Fig. 1.

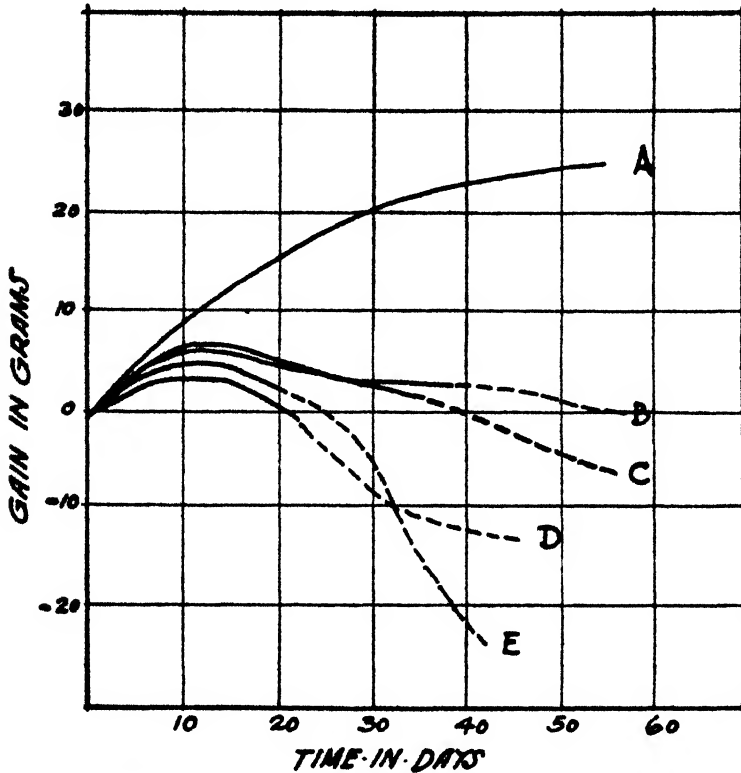


FIG. 1.—Average weight curves of directly comparable groups of experimental animals (rats of initial age of 28 to 29 days) receiving a basal diet devoid of the vitamin B complex but good in all other respects, and in addition: Curve A, 0.4 grams of red kidney beans plus 0.4 grams of autoclaved yeast (daily except Sundays); Curve B, 0.8 grams of red kidney beans (daily except Sundays); Curve C, 0.4 grams of polished rice and 0.4 grams of red kidney beans (daily except Sundays); Curve D, no other food,—these are the negative controls; Curve E, 0.8 grams of autoclaved yeast (daily except Sundays). The average weight curve for each group is represented by a solid line to the point at which the first death occurred among the experimental animals, and from that point onward by a broken line representing the average weight of the surviving experimental animals of the group until the end of the 8 weeks experimental period.

Curve A shows marked supplementation while Curves C and E show deficiencies of vitamin B ( $B_1$ ) of the vitamin complex.

A comparison of these growth curves will show at once that there was no supplementary relationship when the mixture of red kidney beans and polished rice was fed, while, on the other hand, a marked supplementary relationship and not merely an additive effect was shown when the mixture of red kidney beans and the autoclaved yeast was fed. Evi-

dently the vitamin B complex deficiencies of the beans and the rice are not supplemented by each other.

The results obtained in these experiments confirm those of other workers in that autoclaved yeast is devoid of almost all of the vitamin B which was present before autoclaving.

Table I gives a summary of some of the data obtained from the experiments described in this paper. It can be clearly seen with the aid of the

TABLE I  
SUMMARY OF THE DATA OBTAINED FROM THE EXPERIMENTS DESCRIBED IN THIS PAPER

Supplement	No. of Animals	Av. Initial Weight, (Grams)	Av. Net Gains (Grams)	Av. Survival (Days)	Av. Basal Diet Intake (Grams)	Per cent Polyneuritic.
Controls	15	42.9	—11	26	119.9	80
0.8 grams polished rice	20	40.6	—11	50.3	118.8	100
0.8 grams autoclaved yeast	7	35.7	—3.6	26.8	66.4	57
0.8 grams red kidney beans	15	47.6	—5.3	53.4	182.4	40
0.4 grams polished rice plus 0.4 grams autoclaved yeast	10	53.0	—6.4	46.8	188.5	100
0.4 grams red kidney beans plus 0.4 grams autoclaved yeast	12	51.8	25.0	56	263	0
0.4 grams red kidney beans plus 0.4 grams polished rice	23	53.3	—3.5	53.8	174.2	48

growth curves and this table that 0.8 gram of polished rice furnishes very little vitamin B( $B_1$ ) as shown by the number of rats developing polyneuritis, while the 0.8 gram of red kidney beans furnishes a significant amount of this vitamin. The feeding of 0.4 gram each of the red kidney beans and the polished rice did not result in better growth than when the 0.8 gram of red kidney beans was fed alone. This validates the statement that the red kidney beans contain vitamin B( $B_1$ ) since the mixture of the beans and the rice resulted in better growth and in a smaller percentage of cases of polyneuritis than when double the amount of polished rice was fed alone.

The limiting factor of the vitamin B complex in the red kidney beans is vitamin G. This is beautifully demonstrated by the marked growth shown by those animals receiving the mixture of 0.4 gram each of the red kidney beans and autoclaved yeast. The autoclaved yeast is relatively rich in vitamin G in which the red kidney beans are poor as shown by the growth curve resulting when double the weight of the latter is fed alone.

The vitamin B complex of the polished rice is not supplemented by the red kidney beans because the former is deficient in both factors while the latter contains only a relatively low concentration of the vitamin G factor.

#### SUMMARY AND CONCLUSIONS

Experiments are described whereby the limiting factors of the vitamin B complex in red kidney beans and polished rice have been found.

The polished rice was found to be deficient in both factors of the vitamin B complex.

Vitamin G was found to be the limiting factor of the vitamin B complex of red kidney beans which are therefore relatively richer in vitamin B( $B_1$ ) than in vitamin G( $B_2$ ).

The vitamin B deficiency of the polished rice which was used in these experiments is not made good by feeding the rice with red kidney beans, for the former was deficient in both factors while the latter was poor in vitamin G.

#### BIBLIOGRAPHY

1. Sherman, H. C., 1928, *Jour. Nutrition*, I, 191.
2. Chase, E. F., 1928, A Quantitative Study of the Determination of the Antineuritic Vitamin (F, or B). Columbia University Dissertation.
3. Sherman, H. C., and Axtmayer, J. H., 1927, *Jour. Biol. Chem.*, LXXV, 207.
4. Sherman, H. C., and Spohn, A. A., 1923, *Jour. Amer. Chem. Soc.*, XLV, 2719.
5. Osborne, T. B., and Mendel, L. B., 1919, *Jour. Biol. Chem.*, XXXVII, 572.
6. Osborne, T. B., Wakeman, A. J., and Ferry, E. L., 1919, *Jour. Biol. Chem.*, XXXIX, 35.





## THE GASEOUS EXCHANGE OF THE HUMAN SUBJECT I. AS AFFECTED BY INGESTION OF WATER AT 37°C

BY THORNE M. CARPENTER AND EDWARD L. FOX

(*From the Nutrition Laboratory of the Carnegie Institution of Washington,  
Boston, Mass.*)

Received for Publication—September 24, 1929

THE purpose of the experiments reported here was to study the gaseous exchange, the calculated heat production and the carbohydrate metabolized in periods following the ingestion of small quantities of water (50 to 500 cc.) at 37°C. Experiments (to be reported in papers following this one) were made to determine the influence of the ingestion of small amounts of levulose and dextrose. Water at 37°C was given with these sugars with the idea, based upon findings in the previous literature, that the water alone would have little or no effect upon the total metabolism either qualitatively or quantitatively. In order to be certain whether the ingestion of water at body temperature would or would not have an effect upon the total metabolism, the experiments in this paper were made with water alone. In these experiments, as in the sugar experiments, the same routine was observed of basal measurements, a rest period, and measurements extending over several hours following the ingestion of the water.

*Previous investigations.* The effect of the ingestion of water has been studied by Laschtschenko (1898), who observed no change in elimination of carbon dioxide when the temperature of the water was 37°C. Speck (1892) did not report the temperature of the water used in his study. Benedict and Carpenter (1918, p. 140) reported a number of experiments but in none was the water at 37°C. Pollitzer and Stolz (1925) gave lemonade with saccharin, but no temperatures were recorded. Cannon, Querido, Britton and Bright (1927) reported a series of experiments in which warm water was given, but in only one of the experiments was the water nearly 37°C. Their subject A.Q. was given 750 cc. of water at 34.5°C and the maximum increase in heat production was 5.4 per cent 11 minutes after the water was ingested. Lublin (1928) found an increase in the metabolism of from 3 to 8 per cent above the basal value within 2 hours after the ingestion of 800 to 1000 cc. of water (temperature not recorded). Grollman (1929) found 5 to 10 per cent rise in the oxygen consumption within a half hour after the ingestion of 1000 and 1200 cc. of water at 38°.



## METHODS OF INVESTIGATION

The respiratory exchange was determined by the gasometer method in nearly half of the experiments in this paper. The apparatus used were mouthpiece, two Tissot valves, and two Tissot gasometers of 100 liters capacity which were connected in such a way that determinations could be made for a continuous series of periods. Analyses of the expired air were made in duplicate with the Haldane portable apparatus, and usually by two analysts with two of these gas analysis apparatus. Outdoor air was analyzed each morning with each apparatus before it was used for expired air. The basis for repetition of analyses of expired air was a disagreement of more than 0.02 in the respiratory quotients obtained in the duplicate analyses.

The respiratory exchange was also measured by means of the Benedict Universal respiration apparatus, which was used in the same manner as with the chamber respiration apparatus, that is, with duplicate sets of absorbers for carbon dioxide. The change from one period to another was made by shifting the valves controlling the passage of the air through the sets of absorbers. At the end of each period the height at which the spirometer bell stood was noted and in the computations a correction was made for the error due to the compression of the gas in the absorber system and for the change in the position of the spirometer bell during the period. A graphic record of the breathing of the subject, as well as of the oxygen absorption in short periods of time, was obtained by using a kymograph with continuous paper. The tightness of the closed circuit was tested during each period by placing a weight (Benedict 1921) upon the spirometer bell for a certain length of time and noting the slope of the record of the breathing as compared with that obtained when no weight was used.

In both groups of experiments with gasometer and with Universal apparatus, absence of sleep was controlled by applying the method previously described (Benedict and Carpenter, 1918, p. 354; Carpenter, 1925) in which a signal magnet providing a stimulus operated in unison with another signal magnet recording upon the kymograph. The subject was requested to respond to the stimulus by pressing a button which controlled the recording of a third signal magnet.

The general procedure in an experiment was as follows: The subject, J. C., whose age was 43 yrs., height, 167 cms., nude weight, 66 kg., came to the Laboratory without breakfast and lay resting upon a comfortable couch for at least one-half hour. The mouth-piece was then inserted, the nose-clip put on, and measurements of the metabolism were begun

as soon as possible. During the experiment there was usually a light covering over the subject in addition to his clothing and the temperature of the room was not far from 20°C. Six continuous periods, each of 10 to 15 minutes' duration, were first run in order to obtain the basal value for the day. A rest period, varying in length in the different experiments between 15 and 30 minutes, then followed after which the water was given, the quantity having previously been measured and warmed to 37°C. Within one to six minutes after the subject finished drinking the water, the mouthpiece was inserted and observations of the metabolism were resumed in 10 to 15 minute periods which were continuous for 1½ to 2½ hours. A second rest period of from 15 to 30 minutes was taken. The experiment was then concluded with a series of periods, which in most of the experiments was continuous for 1 to 2 hours.

#### RESULTS OF EXPERIMENTS WITH WATER

The water was given by mouth in volumes that varied from 50 to 500 cc. It was taken as quickly as possible, usually by sucking through a small rubber tube. Of the larger quantities (100 cc. or over), all but 25 cc. was first given, and then the 25 cc. for rinsing, in order to imitate as closely as possible the procedure used in the series with dextrose and levulose. The results of the experiments are given in the following tables, the values representing averages for the respiratory quotient, the carbohydrate metabolized, and the heat produced per half hour in the basal periods and in the successive half hours following the ingestion of water.

#### RESPIRATORY QUOTIENTS.

The respiratory quotient is the most important factor measured in this study and in the two succeeding studies, because the object of the experiments with the sugars was to determine the changes in the carbohydrate metabolized when small amounts of either dextrose or levulose were ingested. With the small quantities given it is to be expected that the changes will not be large, and unusual significance therefore has to be attached to slight changes in the respiratory quotient. The breathing appliance used was the mouthpiece. Frequently with untrained subjects this results in distorted breathing, so that the respiratory quotient obtained may not be the true metabolic index. The subject of these experiments was one who had served in hundreds, if not thousands, of periods of observation, in which the mouthpiece was used either with a closed circuit or an open circuit respiration apparatus. He was, therefore, as thoroughly trained to the use of this appliance as one could expect;

TABLE I  
SUMMARY OF RESPIRATORY QUOTIENTS AFTER INGESTION OF WATER

Date and apparatus <sup>1</sup>	Amount of water	Basal <sup>2</sup>	Half hours after ingestion of water								
			1	2	3	4	5	6	7	8	9
1926	cc.										
Jan. 20 U	50	0.88	0.84	0.83	0.86	0.85	0.85	0.85	0.84	0.83	0.83
May 17 U	50	.88	.87	.86	.87	.86	.86	.87	.84	.86 <sup>3</sup>	
Feb. 15 G	50	.80	.81	.80	.80	.85	.79	.79	.79	.79	.78
May 28 G	50	.85	.83	.82	.85	.85	.85	.84	.82	.82	
Average		.86	.84	.83	.85	.85	.84	.84	.82	.83	.81
Jan. 15 U	100	.88	.86	.86	.84	.84	.85	.84	.83	.86	.84
Feb. 8 U	100	.85	.88	.83	.84	.84	.84	.86	.82	.84	.83
Feb. 10 G	100	.85	.86	.87	.86	.86	.85	.84	.83	.85	.83
Apr. 28 G	100	.82	.84	.84	.84	.84	.81	.81	.81	.81	
Average		.85	.85	.85	.85	.85	.84	.84	.83	.84	.83
Jan. 11 U	150	.86	.85	.88	.85	.85	.88	.84	.82	.85	.81 <sup>3</sup>
Apr. 30 U	150	.87	.84	.88	.87	.85	.85	.84	.82	.86 <sup>3</sup>	
Dec. 14 <sup>4</sup> G	150	.83	.84	.83	.83	.82	.83	.82	.82	.81	.81
May 24 G	150	.82	.80	.81	.81	.81	.81	.83	.81	.81	
Average		.85	.84	.84	.84	.84	.84	.83	.82	.83	.81
Jan. 4 U	200	.87	.86	.83	.84	.84	.84	.84	.84	.80	.82
May 12 U	200	.87	.88	.87	.86	.86	.86	.84	.85 <sup>3</sup>	.84	
Nov. 23 <sup>4</sup> G	200	.86	.84	.85	.84	.84	.85	.83	.84	.84	.84
Dec. 1 <sup>4</sup> G	200	.80	.84	.83	.81	.81	.80	.80	.80	.79	.79
Average		.86	.86	.85	.84	.84	.84	.83	.83	.81	.82
Dec. 30 <sup>4</sup> U	250	.82	.80	.78	.80	.80	.82	.81	.81	.80	.81 <sup>3</sup>
Mar. 8 U	250	.83	.84	.84	.84	.84	.84	.81	.82 <sup>3</sup>	.80	
Nov. 16 <sup>4</sup> G	250 <sup>5</sup>	.81	.84	.77	.80	.81	.81	.82	.80	.80	.81
Dec. 23 <sup>4</sup> G	250	.82	.82	.78	.81	.81	.80	.81	.81	.81	.80
May 19 G	250	.81	.82	.82	.81	.81	.81	.81	.80	.81	
Sept. 16 G	250	.84	.84	.85	.84	.84	.84	.84	.82	.82 <sup>3</sup>	
Average <sup>6</sup>		.83	.82	.81	.82	.82	.82	.82	.81	.81	
Dec. 17 G	500	.82	.83	.83	.83	.83	.78	.80	.81 <sup>3</sup>		

<sup>1</sup> U = Universal apparatus; G = Gasometer apparatus.

<sup>2</sup> Average respiratory quotient for five 10-minute periods.

<sup>3</sup> Respiratory quotient for less than a half hour (15 to 28 minutes).

<sup>4</sup> 1925.

<sup>5</sup> Temperature of the water on Nov. 16, 1925 was 16.2°C.

<sup>6</sup> Does not include results of experiment on Nov. 16, 1925.

in fact, he could endure experiments of two hours' duration without interruption. Earlier studies (Carpenter 1915) have shown that the average respiratory quotient obtained with the Universal apparatus agrees with the average obtained with the gasometer apparatus, and later a special study (Hendry, Carpenter and Emmes 1919) showed that another type of closed circuit apparatus gave results agreeing in the main with those obtained with the gasometer apparatus. It is therefore believed that reliance may be placed in the respiratory quotients found.

The respiratory quotients, which represent the total gaseous exchange, are summarized in Table I for the experiments with both the Universal apparatus and the gasometer apparatus.

The determination of the basal metabolism for the day in these experiments differs from the procedure in most of the previous investigations in which short periods have been used. Thus, the average basal figure given in the tables is the result of the measurement of the respiratory exchange in six succeeding periods without interruption, of which the first was rejected because in the majority of instances this differed more from the average value than did any one of the succeeding five. It seemed as though this first period of 10 to 15 minutes was one of adjustment. Since the succeeding five periods were continuous, if one period gave an aberrant quotient, it should be followed by a period in which compensation took place, and it is considered that the average of five succeeding periods measured in this way should come very close to the real basal respiratory quotient. In a similar way the measurement of the respiratory quotient after the interval of rest, whether water was given or sugars were given, was made in an uninterrupted series of periods extending over one and one-half to two hours. The average result for three 10-minute periods in each of the successive hours following the ingestion of water would therefore represent very nearly the true respiratory quotient for a half hour, because opportunity was given for compensation. There were no lapses in these successive measurements except for a short rest period, which occurred about one and one-half hours after the ingestion for the day took place.

As will be seen from Table I, there was a wide variation in the average basal respiratory quotients on the different experimental days, many being high and all of them 0.80 or over. The successive values for periods 2 to 6 on any one day, however, were uniform even when the respiratory quotient was high, as seen in the illustration in Table II, taken from the experiment with the Universal apparatus preceding the ingestion of water on May 17, 1926. An analysis of the basal respiratory quotients

in periods 2 to 6 shows the following: With the Universal apparatus the range of five periods varies from 0.022 on December 30 to 0.077 on January 4; the average range is 0.042. If, however, the best four of the five periods are selected this range is lowered slightly to from 0.054 on January 15 to 0.011 on January 11, May 12, and December 30, and the average range is 0.026. The average range of five periods with the gasometer apparatus is 0.033, and the average range of the selection of the best four is 0.021. The difference between the average quotient with five periods and four periods with the Universal apparatus varies from 0.011 to 0.004, and the average of the average quotients of five periods is 0.868 as compared with

TABLE II  
SAMPLE OF PROTOCOLS OF MEASUREMENTS OF BASAL METABOLISM

Period	Time	Duration of period	CO <sub>2</sub> per minute	O <sub>2</sub> per minute	Respiratory quotient
		min.	cc.	cc.	
1	9.02-a.m.	10.33	200	209	0.96
2	9.12- "	11.42	190	204	.93
3	9.23- "	10.78	189	210	.90
4	9.34- "	11.00	192	208	.92
5	9.45- "	10.78	189	208	.91
6	9.56- "	11.45	191	211	.91

0.867 for four periods. With the gasometer apparatus the difference between the average of five periods and the average of four periods is less than 0.010 in all cases, and the average quotient is identical when all the experiments are taken into consideration. In general, then, it does not make any difference whether four periods or five periods are selected for the average basal quotient. In other words, there is a high degree of uniformity in the five successive measurements which are selected for the average basal quotient. Therefore, small differences in the average respiratory quotient from half hour to half hour are of significance. If there was actually a change, for example, in the respiratory quotient over basal of 0.01, and this persisted for several periods, the change is considered to be significant.

#### METABOLISM OF CARBOHYDRATE

The respiratory quotients were computed from measurements of total carbon dioxide and oxygen, which include the amounts resulting from the metabolism of protein. For the computation of total heat production, and consequently the metabolism of carbohydrate, the respiratory ex-

change was apportioned to the different nutrients, the metabolism of protein being calculated by means of the necessary factors and from the average urinary nitrogen for this series (0.47 gram per hour in the experiments with the Universal apparatus and 0.40 gram per hour in the experiments with the gasometer). The amounts of carbohydrate metabolized as calculated for the basal periods and for the half hours following the ingestion of water are given in Table III.

In the experiments with the smaller quantities of water, 50 and 100 cc., there were usually lower respiratory quotients in the half hours following the ingestion of the water than in the basal periods, the decreases beginning for the most part in the first or near the first half hour. The greater decreases in the quotients occurred after the higher basal quotients. The average variations for the half hours in the two groups (50 cc.,  $-0.03$ ; 100 cc.,  $\pm 0.01$ ) give some indication of the tendency to small variation or actual decrease in the respiratory quotients following the ingestion of small amounts of water.

Following the ingestion of the smallest amount (50 cc.) of water there was a decreased metabolism of carbohydrate in comparison with basal metabolism in all but two of the half-hour periods, the two exceptions being  $+0.07$  and  $+0.05$  gram in the first and second half hours on January 20 and February 15, respectively. The largest decrease followed high basal carbohydrate accompanying high basal respiratory quotient.

Experiments with 100 cc. of water gave results somewhat different from those obtained with 50 cc. of water, even though there was high basal carbohydrate (respiratory quotient, 0.88) in one of the four experiments, for in all the experiments there was increased metabolism of carbohydrate in the first half hour and two of the experiments showed increases in other half hours. The net changes in 4 hours ranged from  $+1.0$  gram to  $-4.7$  grams.

Among the four experiments with 150 cc. of water, the first two were experiments with high basal respiratory quotients, but in neither of these was there a marked change from the basal value until the sixth and seventh half hours ( $-0.05$  and  $-0.07$ ) after the ingestion of water, so that in relation to high basal quotients there was a delayed drop in the respiratory quotient after this amount of water as compared with the drop in quotient after the smaller quantities of water. The maximum change in the other two experiments, on December 14 and May 24 with normal basal quotients, was  $-0.02$  and on May 24 there was a practically constant respiratory quotient for about five hours. The ingestion of 150 cc. of water was followed by decreased metabolism of carbohydrate in

TABLE III  
SUMMARY OF CARBOHYDRATE METABOLIZED AFTER INGESTION OF WATER (Grams per half hour).

Date and apparatus <sup>1</sup>	Amount of water	Basal <sup>2</sup>	Half hours after ingestion of water								
			1	2	3	4	5	6	7	8	9
1926 Jan. 20 U May 17 U Feb. 15 G May 28 G	cc.	3.76	3.83	2.67	2.48	3.08	2.86	2.92	2.52	2.33	2.59
	50	4.55	3.80	3.51	3.29	3.40	3.21	3.53	2.52	3.19*	
	50	2.25	2.08	2.30	2.05	1.83	1.61	1.59	1.60	1.61	1.61
	50	3.12	2.94	2.51	2.49	2.89	3.06	2.65	2.08	2.30	
	Average	3.42	3.16	2.75	2.58	2.80	2.69	2.67	2.18	2.36	2.10
Jan. 15 U Feb. 8 U Feb. 10 G Apr. 28 G	100	3.64	3.99	3.17	3.00	2.67	3.19	2.77	2.51	3.08	2.92
	100	3.25	3.92	3.03	2.65	2.75	2.81	3.37	2.84	2.81	2.68
	100	3.13	3.39	3.64	2.71	3.35	3.17	2.71	2.50	3.19	2.52
	100	2.30	2.76	2.81	2.71	2.73	2.05	2.08	2.10	2.13	
	Average	3.08	3.52	3.11	3.00	2.88	2.81	2.73	2.49	2.66	2.71
Jan. 11 U Apr. 30 U Dec. 14 <sup>3</sup> G May 24 G	150	4.24	3.19	3.45	3.62	3.08	3.64	2.52	2.18	2.93	2.07*
	150	3.49	3.49	2.75	2.88	3.43	3.05	2.67	2.35	3.00*	
	150	2.95	2.50	2.84	2.59	2.59	2.52	2.32	2.35	1.95	2.16
	150	2.54	2.24	1.90	2.16	2.09	2.13	2.65	2.25	2.25	
	Average	3.31	2.86	2.74	2.81	2.80	2.84	2.54	2.28	2.53	2.12
Jan. 4 U May 12 U Nov. 23 <sup>4</sup> G Dec. 1 <sup>5</sup> G	200	4.73	4.19	3.67	2.62	3.06	3.13	2.98	3.09	1.97	2.44
	200	3.49	3.62	3.69	3.64	3.19	3.37	2.60	3.21*	2.71	2.73
	200	3.27	3.33	2.79	2.99	2.71	3.10	2.49	2.70	1.72	1.94
	200	2.13	2.08	3.12	2.88	2.36	1.93	2.16	2.19	1.72	
	Average	3.41	3.46	3.32	3.03	2.83	2.88	2.56	2.80	2.13	2.37
Dec. 30 <sup>4</sup> U Mar. 8 U Nov. 16 <sup>4</sup> G Dec. 23 <sup>4</sup> G May 19 G Sept. 16 G	250	1.93	2.58	1.98	1.33	1.85	2.27	2.38	2.15	1.88	2.08*
	250	2.54	3.01	2.79	3.01	2.79	2.79	2.18	2.19*	1.92	1.90
	250*	1.93	2.26	2.82	1.39	1.96	1.94	2.32	1.75	2.08	1.72
	250	2.08	2.08	2.32	1.27	2.12	1.86	2.09	2.07	2.08	
	250	2.21	2.30	2.46	2.25	2.25	2.21	2.25	2.19	2.19	
	250	3.70	3.19	3.14	3.11	3.09	2.90	2.93	2.27	2.51*	
	Average <sup>5</sup>	2.49	2.63	2.55	2.24	2.42	2.41	2.37	2.09	2.17	
Dec. 17 G	500	1.90	2.43	2.62	2.98	2.78	1.45	1.86	2.13*		

<sup>1</sup> U = Universal apparatus; G = Gasometer apparatus.

<sup>2</sup> Calculated to one half hour from five 10-minute periods.

<sup>3</sup> Calculated from results for less than a half hour (15 to 28 minutes).

<sup>4</sup> 1925.

<sup>5</sup> Temperature of the water on Nov. 16, 1925 was 16.2°C.

\* Does not include results of experiment on Nov. 16, 1925.

practically all half hours, whether the basal carbohydrate was high or comparatively low, the decreases averaging for the four experiments 1.28 to 0.33 grams per half hour and the total decrease in 4 hours ranging from 9.31 (basal carbohydrate, 4.24 grams and respiratory quotient, 0.89) to 2.65 (basal carbohydrate, 2.54 grams and respiratory quotient, 0.82). In only one first half hour was there no change and in a single sixth half hour there was an increase of 0.11 gram.

There were three experiments of the four with 200 cc. of water in which the basal respiratory quotient was high; the quotient in the fourth experiment was normal. One of the high basal quotients was 0.90 on January 4 and from this quotient there was a marked drop to quotients lower by 0.07, 0.10 and 0.08 in the third, eighth and ninth half hours, with an average variation of  $-0.06$ . In the other two experiments with high basal quotient, the variations after the ingestion of water were low, averaging  $-0.01$  and  $-0.02$ , and in the experiment with the normal basal quotient, there was an increased quotient in the first four half-hour periods. In most of the experiments, then, 200 cc. of water delayed or prevented the fall in respiratory quotient that occurred after the smaller quantities of water. In two of the four experiments with 200 cc. of water, there were increases in carbohydrate metabolized in the first two half hours and in one of these two experiments, the increase continued throughout the first four half hours. There is some indication that, with increase in the amount of water ingested, there is less decrease from the basal carbohydrate metabolized and there is even increase in the metabolism of carbohydrate.

In only one of the six experiments with 250 cc. of water was there a high basal quotient, *i.e.*, 0.87 on September 16, from which quotient the variations in the half hours after the ingestion of water were from  $-0.02$  in the third half hour to  $-0.05$  in the seventh and eighth half hours. The other five experiments, including the experiment on November 16, 1925, with water at  $16.2^{\circ}\text{C}$ ., showed very small changes from basal respiratory quotients that were all normal, the maximum variation being  $-0.04$  on two different days. In three of the experiments, the quotient was higher ( $+0.03$  in one instance) in the second half hour than the basal quotient and in all five the quotient in the first half hour was either slightly higher than the basal quotient or not changed. In each of these five experiments with basal quotients from 0.80 to 0.83, there was a practically constant respiratory quotient for a period of 5 to 6 hours.

Of the six experiments (including the experiment of Nov. 16, 1925, with water at  $16.2^{\circ}\text{C}$ .) there were five in which there was either little or no



decrease in carbohydrate metabolized or else an increase in the first two and one-half hours. This increase persisted in two instances to three and one-half hours.

The single experiment with 500 cc. of water gave results following a normal basal quotient which are somewhat similar to the results in five of the experiments in the preceding group. There was a slightly increased respiratory quotient in the first four half hours after the ingestion of water. There was an increase in metabolism of carbohydrate in the first four half hours above the basal carbohydrate of 1.9 grams (respiratory quotient 0.81) and a net increase in 3 1/2 hours of 3.0 grams.

In general there was the greatest falling off in the respiratory quotient after the ingestion of water when the basal respiratory quotient was high (the higher the initial quotient the greater the potentiality for a fall) and the larger the quantity of water ingested the less was the decrease in the respiratory quotient in the succeeding half hours.

There was a decrease in the amount of carbohydrate burned in the periods subsequent to the ingestion of 50 cc. of water, whether there was a low or high basal metabolism of carbohydrate. When the quantities of water were 100 cc. or over, there were in some cases increases in carbohydrate above the basal amount and in others there were decreases, the increases being more marked after the higher quantities of water, and to a certain extent independent of the height of the basal carbohydrate and the basal respiratory quotient. Stated more exactly, there was increased metabolism of carbohydrate when the water was 200 cc. or over, both in comparison with the results of ingestion of smaller amounts of water and in comparison with the basal carbohydrate.

#### HEAT PRODUCTION

The heat production calculated per half hour for the basal condition and for the half hours after the ingestion of varying quantities (50 to 500 cc.) of water at 37° C. is given in Table IV. The average basal value was 31.1 calories, the maximum (on January 4) being 2.5 calories above the average, that is, 8 per cent. The minimum (on January 15) was 29.5 calories, that is, 5 per cent below the average, so that in the 23 experiments with water at 37° C. there was a range of 13 per cent in the basal heat production.

The heat production after 50 cc. of water showed an increase above the basal value in the first half hour in each of the four experiments, but in three experiments it was progressively lowered during the remainder of four hours after the ingestion of water, the net change in 4 hours being

-4.9, -6.6 and -2.1 calories in the respective experiments. In one experiment, the heat production was increased 1.1 calories in the first hour and then remained practically without change during the remainder of the experiment.

The ingestion of 100 cc. of water, with the exception of the experiment on February 10, was followed for the most part by small changes in heat production of which the largest were +0.9 in the fifth half hour on January 15, -0.9 in the fourth half hour on February 8 and +1.0 calorie in the second half hour on April 28. On February 8 the values decreased after the first half hour and the net change in 4 hours from the basal value was -1.8 calories; on April 28 the values were mostly increased, the total increase being 2.0 calories. In the experiment on February 10, the basal value was high and though there was a slight increase in heat production in the first half hour after the ingestion of water, there were significant decreases in all the succeeding half hours, the maximum decrease being 1.9 calories; the net change in 4 hours was -9.5. It may be questioned whether 32.3 calories was the real basal value for this experiment in view of the marked decrease in all but one of the subsequent periods.

In the experiments with 150 cc. of water, the heat production decreased in comparison with the preceding basal heat production, whether the basal values were below or above the average basal value for all the experiments with water. On April 30, there were but slight changes from the basal value, 29.7 calories, throughout the experiment, and there was the small change of -2.2 calories in 4 hours. The high basal values on December 14 and May 24, 32.7 and 32.9 calories, were followed in all subsequent half hours by decreases, many of them marked, of which the maximum was 2.6 calories in the fourth hour on May 24. The total decrease in 4 hours in the two experiments was 11.7 and 12.3 calories. As in the experiment on February 10 with 100 cc. of water, however, the high basal values were a possible cause of these large decreases and it may be questioned whether they are actually significant. The basal value on January 11 was close to the average basal value, so that the decreases in this experiment seem significant.

Among the four experiments with 200 cc. of water there were two in which the basal value was high, namely, on January 4 and December 1. Nevertheless, there was on January 4 a rise in heat production in the first hour after the ingestion of water, a gradual falling off in the remaining half hours with no greater decrease than 1.4 calories and a net change of but -3.2 calories in 4 hours, and on December 1, an increase in practically all of the half hour periods after the ingestion of water and a total

TABLE IV  
SUMMARY OF HEAT PRODUCTION AFTER INGESTION OF WATER. (Calories per half hour)

Date and apparatus <sup>1</sup>	Amount	Basal <sup>2</sup>	Half hours after ingestion of water.								
			1	2	3	4	5	6	7	8	9
1926 Jan. 20 U May 17 U Feb. 15 G May 28 G	cc.										
	50	30.2	30.7	29.2	29.2	29.5	29.3	29.7	29.6	29.5	30.2
	50	30.3	30.5	29.9	29.7	29.2	29.1	28.8	29.6	29.0 <sup>3</sup>	32.2
	50	32.2	32.8	32.7	32.4	32.5	32.2	32.0	32.1	32.4	32.2
	50	30.5	30.7	30.4	30.3	30.2	30.0	29.9	30.1	30.3	
Jan. 15 U Feb. 8 U Feb. 10 G Apr. 28 G	Average	30.8	31.2	30.6	30.4	30.4	30.2	30.1	30.4	30.3	31.2
	100	29.5	29.3	30.2	28.9	29.2	30.4	30.1	29.4	29.5	29.7
	100	30.8	31.3	30.6	30.8	29.9	30.4	30.3	30.7	30.6	31.0
	100	32.3	32.7	31.4	31.3	30.7	30.9	30.5	30.4	31.0	30.6
	100	30.3	30.9	31.3	30.5	30.6	29.9	30.1	30.4	30.7	
Jan. 11 U Apr. 30 U Dec. 14 <sup>4</sup> G May 24 G	Average	30.7	31.1	30.9	30.4	30.1	30.4	30.3	30.2	30.5	30.4
	150	30.8	30.4	29.4	29.4	29.5	29.5	29.6	29.9	29.9	30.9 <sup>3</sup>
	150	29.7	29.7	29.9	29.4	29.3	29.3	29.2	29.7	28.9 <sup>3</sup>	31.2
	150	32.7	32.5	31.6	31.3	31.3	30.6	30.6	30.9	31.1	
	150	32.9	32.0	30.4	31.2	30.3	30.7	31.9	32.2	32.2	
Jan. 4 U May 12 U Nov. 23 <sup>5</sup> G Dec. 1 <sup>6</sup> G	Average	31.5	31.2	30.3	30.3	30.1	30.0	30.3	30.7	30.5	31.1
	200	34.5	34.5	34.0	32.4	32.6	33.2	33.8	32.9	32.2	32.8
	200	30.6	30.6	29.8	30.8	30.4	30.3	30.3	30.5 <sup>3</sup>	30.5	30.6
	200	30.1	30.5	31.2	31.1	30.5	30.3	30.3	30.3	30.5	30.6
	200	33.5	34.5	34.3	34.2	33.5	33.9	33.8	34.3	34.2	34.0
Dec. 30 <sup>4</sup> U Mar. 8 U Nov. 16 <sup>5</sup> G Dec. 23 <sup>6</sup> G May 19 G Sept. 16 G	Average	31.7	32.5	32.3	32.1	31.8	31.9	32.1	32.0	32.3	32.5
	250	31.7	32.0	32.4	32.0	30.7	30.9	32.1	31.9	31.1	31.0 <sup>3</sup>
	250	29.7	30.4	30.2	30.2	30.2	30.2	30.9	30.0 <sup>3</sup>	30.7	30.4
	250 <sup>5</sup>	30.8	32.3	31.5	32.2	31.2	30.9	30.6	31.2	30.7	30.7
	250	30.1	30.1	30.6	30.0	30.6	29.9	30.3	30.0	30.1	
Dec. 17 G	250	31.7	32.7	32.5	32.1	32.2	31.7	32.2	31.7	31.4	
	250	31.8	32.9	32.4	32.1	32.0	32.2	32.5	32.4	32.7 <sup>3</sup>	
	Average <sup>3</sup>	31.0	31.6	31.6	31.3	31.1	31.0	31.4	31.2	31.3	
	500	30.4	31.8	31.6	33.0	33.2	29.7	29.9	30.7 <sup>3</sup>		

<sup>1</sup> U = Universal apparatus; G = Gasometer apparatus.

<sup>2</sup> Calculated to one half hour from five 10-minute periods.

<sup>3</sup> Calculated from results for less than a half hour (15 to 28 minutes).

<sup>4</sup> 1925.

<sup>5</sup> Temperature of the water on Nov. 16, 1925 was 16.2°C.

<sup>6</sup> Does not include results of experiment on Nov. 16, 1925.

increase of 4.7 calories in 4 hours. In the two experiments on May 12 and November 23, the basal values were about 3 per cent lower than the average basal value in all the experiments with water and in all the succeeding half hour periods there was a small increase in heat production, an average of 0.7 calorie per half hour on May 12 and 0.4 calorie on November 23. In this group of experiments, three out of four showed a definite increase in heat production as the result of the ingestion of 200 cc. of water at 37° C.

In the group of six experiments with 250 cc. of water, which includes the experiment on November 16, 1925 with water at 16.2° C. there were four (March 8, November 16, May 19 and September 16) which gave increases in heat production for all, or nearly all, of the half hour periods after the ingestion of water. The increases are small (maximum 1.5 calories for water at 37° C.) but they seem significant because they are so numerous and because the group with 200 cc. of water indicated the same tendency. There was a negative change in 4 hours in only one of the six experiments, that is, 0.5 calorie on December 30. It is a striking fact that 200 cc. and 250 cc. of water at 37° C. have an effect on the metabolism, which cannot be ascribed to low basal metabolism because it has been shown that increases in heat production in both groups bear no relation to the basal values. In the one experiment with 500 cc. of water, the basal value was not far from the general average. There was a significant increase in the heat production for the first four half hours after the ingestion of water and a total change of +7.1 calories in three and one half hours.

In general, then, heat production after the ingestion of water at 37° C. was increased when the quantities were at least 200 cc. Some of the experiments with amounts of water smaller than 200 cc. gave slight increases in the heat production, but in most instances there was little or no change. In those experiments in which there was a marked decrease, it followed for the most part a high basal heat production. This was particularly true on February 10 with 100 cc. of water and on December 14 and May 24 with 150 cc. of water. In all the subsequent experiments in the table, in which there was a high basal value, there occurred either a positive increase for the early portion of the experiment or an increase for all of the experiment.

#### RESULTS OF CONTROL EXPERIMENTS WITHOUT WATER

To determine the course of the metabolism without water, six control experiments were made with the same subject, J. C., in the post-absorptive condition, three with the Universal respiration apparatus and three

by the gasometer method, the order, number, and length of the periods of measurement without water being the same as in the experiments with water. In these control experiments there was a tendency for the respiratory quotients to be lower in the half hours following the preliminary and the rest periods and for the metabolism of carbohydrate to be lower, whether the preliminary respiratory quotient was normal or high. The heat production, as averaged for the different half hours, showed more increase than decrease in comparison with the preliminary heat production, although in the greater number of individual half hours of the experiments there were decreases which were generally small. These results when the subject was given no water are similar to the results found in the experiments with the smaller amounts of water.

#### GENERAL DISCUSSION

It has been shown in the preceding discussion that when the quantities of water are large enough, there is a distinct increase in the carbohydrate metabolized as compared with the basal value, and also in the heat production. There seems to be, however, no quantitative relation between the increase in carbohydrate metabolized and the increase in heat production. That is to say, the increase in heat production cannot be accounted for mathematically by the heat of combustion of the additional carbohydrate metabolized. In general when there is a fall in the carbohydrate metabolized there is also a fall in the heat production, but in some groups the fall in carbohydrate metabolized is much greater with respect to its energy content than is the fall in total heat production. For example, in 4 hours with 150 cc. of water there was a lowering of the carbohydrate metabolized by 5 grams, which would be approximately 19 calories. But there was a fall of only 9 calories in the heat production. The values given for heat production are calculated from the respiratory exchange, and therefore increases in heat production are real increases in total metabolism.

Lublin (1928) accounts for the increased metabolism after ingestion of water as due to the work performed by the kidneys. Grollman (1929), however, points out that after the ingestion of water the rise in oxygen consumption is rapid, but that the metabolic change does not run parallel to the diuresis. He believes that the diuresis and metabolic rise are not related, but that the latter results from a general stimulation of the metabolism of the body cells, which may be considered as due to the dilution of the internal environment of the cells by the ingested fluid. In none of our experiments was there a marked diuresis, and it is questionable

whether the increased metabolism is due primarily to increased kidney activity or is due, first, to the transfer of the fluid from the alimentary tract into the blood stream, from which subsequently it is either removed by the tissues or secreted by the kidneys.

The findings suggest strongly that the transfer of fluid in the body, even when at body temperature, involves expenditure of energy. Also, the results in general indicate that the greater the quantity of water, the greater the requirement of energy, although one group of experiments (150 cc.) gave anomalous results in that both the metabolism of carbohydrate and the heat production fell off markedly compared with the results obtained in the other groups. There is no satisfactory explanation for this exception. As will be seen in subsequent papers, the same sort of phenomenon occurred in the experiments with dextrose and with levulose.

#### SUMMARY

A series of experiments was made with a human subject, in which the respiratory exchange in the post-absorptive state was measured for a period of one hour before, and for  $3\frac{1}{2}$  to  $4\frac{1}{2}$  hours after, the ingestion of 50 to 500 cc. of water at  $37^{\circ}\text{C}$ . A few control experiments without water, under conditions otherwise similar, were also carried out. The measurements of the metabolism were made by means of the Benedict Universal apparatus and by use of the gasometer method.

In the control experiments without water, and in the experiments with ingestion of water in amounts less than 200 cc., the course of the carbohydrate metabolism and the heat production changed little, if at all.

The ingestion of 250 cc. of water was followed by a slight, but definite increase in the metabolism of carbohydrate for a period of one hour (average 4 per cent), and with 500 cc. there was an increase (42 per cent) lasting two hours. Thus amounts of water over 200 cc. work for a definite increase in the metabolism of carbohydrate.

The heat production was slightly increased (2 per cent) for  $1\frac{1}{2}$  hours in experiments with 200 cc. of water, for two hours (1 per cent) with 250 cc. of water and for two hours (7 per cent) with 500 cc. of water at  $37^{\circ}\text{C}$ .

#### BIBLIOGRAPHY

- Benedict, F. G., 1921, *Jour. Amer. Med. Assoc.*, LXXVII, 247.  
Benedict, F. G., and Carpenter, T. M., 1918, *Carnegie Institution of Washington, Pub. No. 261*.  
Cannon, W. B., Querido, A., Britton, S. W., and Bright, E. M., 1927, *Amer. Jour. Physiol.*, LXXIX, 466.  
Carpenter, T. M., 1915, *Carnegie Institution of Washington, Pub. No. 216*, 150.

Carpenter, T. M., 1925, *Carnegie Institution of Washington, Pub. No. 369*, 25.

Grollman, A., 1929, *Amer. Jour. Physiol.*, LXXXIX, 159.

Hendry, M. F., Carpenter, T. M., and Emmes, L. E., 1919, *Boston Medical and Surgical Jour.*, CLXXXI, 285.

Laschtschenko, P., 1898, *Arch. f. Hygiene*, XXXIII, 145.

Lublin, A., 1928, *Zeit. f. klin. Med.*, CIX, 371.

Pollitzer, H., and Stolz, E., 1925, *Wien. Arch. f. innere Med.*, XI, 341.

Speck, C., 1892, *Physiologie des menschlichen Atmens, nach eigenen Untersuchungen*, Leipzig, 42.



## THE GASEOUS EXCHANGE OF THE HUMAN SUBJECT II. AS AFFECTED BY SMALL QUANTITIES OF DEXTROSE

BY THORNE M. CARPENTER AND EDWARD L. FOX

*(From the Nutrition Laboratory of the Carnegie Institution of  
Washington, Boston, Mass.)*

Received for Publication—September 24, 1929

**I**N CONNECTION with an earlier investigation in this Laboratory (Carpenter 1925) an experiment was reported on the effect of 30 grams of dextrose on the respiratory exchange. The change in the respiratory quotient was so large, that questions arose as to how small a quantity of dextrose would produce significant changes in the respiratory quotient and metabolism, whether the type of reaction would be the same with all quantities, and whether the increases in carbohydrate metabolized and heat produced would bear a definite relationship to the amounts of dextrose ingested.

Although the effect of dextrose upon the respiratory exchange has been reported many times, there are few studies with man in which small quantities (under 50 grams) have been used. Mason (1925) determined the respiratory quotient and calculated the heat production after the ingestion of 25 grams of dextrose. He found no rise above the basal respiratory quotient, but an increase of 10 per cent in the heat production at the end of one hour after ingestion of the sugar. Lublin (1926) reported one experiment with 30 grams and two experiments with 38 grams. In all three there was a rise in respiratory quotient and oxygen absorption within one-half to one hour. Lambie and Redhead (1929) had one experiment with a normal subject to whom 20 grams of glucose were given intravenously, and the gaseous exchange was measured. The maximum respiratory quotient was found twenty minutes after the beginning of injection.

### THE METABOLISM AS AFFECTED BY SMALL QUANTITIES OF DEXTROSE

The results of our measurements of the respiratory exchange before and after the ingestion of small amounts of dextrose in water are given in Tables I to IV. The values are averages per half hour for the respiratory quotient, carbohydrate metabolized and heat production during the basal period and during the successive half-hours after the dextrose was taken. The methods of studying the respiratory exchange were the same as in the previous communication (*This Jour.* 1930, II, 360.). The



TABLE I  
SUMMARY OF RESPIRATORY QUOTIENTS AFTER INGESTION OF DEXTROSE

Date and apparatus <sup>1</sup>	Amount ingested		Basal <sup>2</sup>	Half hours after ingestion of dextrose.														
	Dextrose	Water		1	2	3	4	5	6	7	8	9						
1926 May 26 U June 2 U Feb. 17 G Feb. 24 G	grams	cc.	0.85	0.86	0.87	0.87	0.87	0.87	0.87	0.87	0.86	0.85	0.81					
	5.05	50	.81	.83	.81	.83	.82	.81	.82	.81	.81	.81	.80	0.81				
	5.05	50	.81	.81	.83	.83	.82	.82	.82	.81	.81	.80	.80	.80				
	5.25	50	.79	.80	.83	.83	.80	.80	.80	.80	.80	.78	.78	.81				0.78
	5.25	50																.80
		Average	.82	.83	.84	.84	.83	.82	.83	.82	.82	.81	.81	.81				
Apr. 26 U Feb. 19 U Mar. 1 G Mar. 5 G	10.1	100	.89	.91	.90	.89	.90	.89	.90	.89	.87	.85	.85	.85				
	10.5	100	.81	.83	.80	.83	.80	.81	.81	.82	.81	.82	.80	.79				.81
	10.5	100	.83	.82	.87	.88	.86	.85	.86	.85	.83	.83	.83	.81				.81
	10.5	100	.83	.83	.84	.87	.86	.83	.82	.83	.82	.80	.83	.83				.83
		Average	.84	.85	.85	.87	.86	.85	.83	.83	.82	.82	.82	.82	.82			
Mar. 26 U Feb. 26 U Apr. 23 G Mar. 10 G	15.5	150	.85	.86	.87	.91	.89	.88	.87	.88	.87	.85	.82	.83				.85
	15.75	150	.87	.88	.91	.91	.88	.87	.88	.87	.88	.87	.86	.86				.82
	15.5	150	.79	.79	.82	.83	.83	.81	.79	.79	.79	.79	.79	.79				.85
	15.75	150	.83	.84	.88	.87	.87	.86	.84	.82	.82	.82	.82	.82				.83
		Average	.84	.84	.87	.88	.87	.86	.85	.83	.83	.82	.82	.82	.82			
Mar. 3 U Apr. 15 U May 3 G Mar. 15 G	21	200	.84	.87	.89	.90	.90	.88	.87	.87	.87	.85	.83	.84				.83
	21	200	.84	.86	.86	.87	.87	.87	.87	.87	.87	.85	.83	.84				.81
	21	200	.81	.83	.87	.88	.87	.85	.87	.87	.87	.82	.81	.81				.83
	20	200	.84	.86	.91	.90	.88	.89	.88	.87	.87	.85	.85	.85				.83
	21	200																
	Average	.83	.86	.88	.89	.88	.88	.87	.86	.85	.84	.84	.82	.82				.82
Jan. 25 U Mar. 31 U Mar. 19 G May 14 G	25	250	.89	.90	.91	.93	.92	.89	.90	.89	.90	.89	.89	.89				.87 <sup>2</sup>
	26	250	.81	.83	.87	.89	.85	.82	.84	.82	.84	.83	.82	.82				.80 <sup>2</sup>
	26	250	.86	.84	.89	.90	.90	.88	.85	.85	.85	.82	.83	.83				.83
	26	250	.82	.82	.87	.87	.86	.86	.83	.83	.83	.83	.81	.81				.81 <sup>2</sup>
		Average	.85	.85	.89	.90	.88	.88	.86	.86	.86	.86	.83	.83	.83			
Feb. 12 U Dec. 10 G Dec. 15 G	35.8	350	.85	.84	.87	.90	.95	.89	.88	.87	.88	.87	.89	.89				.83 <sup>2</sup>
	52	500	.80	.83	.89	.91	.90	.88	.90	.89	.89	.89	.82 <sup>2</sup>	.82 <sup>2</sup>				
	104	500	.81	.84	.90	.91	.90	.90	.94	.92	.92	.92	.92	.92				

<sup>1</sup> U = Universal apparatus; G = gasometer apparatus.<sup>2</sup> Average for 50 minutes.<sup>3</sup> Average for less than a half hour (15-28 minutes).

dextrose was Kahlbaum's purified grape sugar. This was weighed, dissolved in water, warmed to 37° C. and taken through a rubber tube; 25 cc. pure water being used as final rinse water taken in the same manner.

#### RESPIRATORY QUOTIENT AND METABOLISM OF CARBOHYDRATE

The respiratory quotients calculated by half hour periods are given in Table I for the Universal apparatus and the gasometer method. As in the series with small quantities of water, the basal quotient varied widely, ranging from 0.79 to 0.89, but in none were there such low quotients as are occasionally seen in the literature, *viz.*, under 0.75. The changes after the ingestion of dextrose vary with the initial basal quotient and with the quantity of dextrose given. The amount of carbohydrate metabolized before and after ingestion of dextrose is given in Table II. In calculating the non-protein respiratory quotient correction was made for protein, using an average of 0.38 gram of nitrogen per hour. During the basal hour the amounts of carbohydrate varied from 1.68 to 3.99 grams per half hour, and for the most part were over 2 grams.

In the 4 experiments with 5 grams in 50 cc. of water, there were in general during the first two hours slight increases in the respiratory quotient, the greatest occurring in the experiment on February 24 in the third half hour, 0.04. There was a positive increase in the carbohydrate metabolized in three of the experiments for the first two hours, and in two, through the third hour. The increase in carbohydrate burned was greatest with the lowest basal figure, that is, on February 24. The reverse rule, however, does not hold as the next greatest increase in any experiment occurs with the highest basal carbohydrate metabolized on May 26. The maximum average summation of increases<sup>1</sup> was 0.8 gram, equivalent to 16 per cent of the amount ingested.

Ten grams of dextrose in 100 cc. of water were given in 4 experiments. There was a rise in the respiratory quotient in the majority of the periods for the first two hours. In the experiment on March 1, in the third half hour, there was an increase of 0.05. The first two experiments show an irregularity in the carbohydrate metabolized from one half hour to the next. On March 1, with the exception of the first half hour, there was an increase in the carbohydrate metabolized during the first 2½ hours with a subsequent lowering during the last of the experiment, that is, from the

<sup>1</sup> By this expression is meant the greatest increase that can be obtained mathematically by adding the increases in *successive half* hours. The number of half hours so used will vary in the different experiments according to the duration of increases as well as the amount of dextrose ingested.

TABLE II  
SUMMARY OF CARBOHYDRATE METABOLIZED AFTER INGESTION OF DEXTROSE (Grams per half hour)

Date and apparatus <sup>1</sup>	Amount ingested		Basal <sup>2</sup>	Half hours after ingestion of dextrose												
	Dextrose	Water		1	2	3	4	5	6	7	8	9				
				grams	cc.											
1926																
May 26 U	5.05	50	3.17	3.36	3.36	3.66	3.29	3.50	3.33	3.03						
June 2 U	5.05	50	2.39	2.53	2.53	2.08	2.51	2.13	2.12	1.92	2.38					
Feb. 17 G	5.25	50	2.44	2.32	2.21	2.73	2.38	2.14	2.18	1.98	1.97					
Feb. 24 G	5.25	50	1.68	1.95	2.00	2.70	2.12	1.88	2.13	1.50	2.12					1.57
		Average	2.42	2.56	2.53	2.79	2.58	2.41	2.44	2.11	2.16					1.92
Apr. 26 U	10.1	100	3.99	4.45	4.24	3.91	4.06	3.94	3.37	3.08	3.03					
Feb. 19 U	10.5	100	2.19	2.74	2.16	2.93	2.04	2.20	2.18	2.42	1.56					2.14
Mar. 1 G	10.5	100	2.76	2.62	3.84	3.91	3.17	2.95	2.62	2.67	2.30					2.30
Mar. 5 G	10.5	100	2.43	2.76	3.02	3.66	3.17	2.67	2.49	1.83	2.57					2.59
		Average	2.84	3.14	3.32	3.60	3.11	2.94	2.67	2.50	2.37					2.34
Mar. 26 U	15.5	150	3.01	3.48	3.56	4.48	3.86	3.71	3.35	2.90	2.62					2.49
Feb. 26 U	15.75	150	3.56	4.01	4.77	4.83	3.83	3.41	3.62	3.37	3.23					3.03
Apr. 23 G	15.5	150	1.79	1.87	2.47	2.45	2.68	2.10	1.74	1.97	2.03					
Mar. 10 G	15.75	150	2.70	3.09	4.14	3.84	3.64	3.44	2.86	2.53	2.59					2.82
		Average	2.77	3.11	3.74	3.90	3.50	3.17	2.89	2.69	2.62					2.78
Mar. 3 U	21	200	2.91	3.66	4.45	4.40	3.99	3.64	3.40	3.03	2.84					2.67
Apr. 15 U	21	200	2.71	3.52	3.83	3.76	3.60	3.71	3.69	2.78	2.96					2.22
May 3 G	20	200	2.40	3.06	4.10	3.73	3.84	3.22	2.61	2.68	2.52					
Mar. 15 G	21	200	3.13	3.74	5.03	4.50	3.80	4.03	3.66	3.31	3.17					2.79
		Average	2.79	3.50	4.35	4.10	3.81	3.65	3.34	2.95	2.87					2.56
Jan. 25 U	25	250	3.87	4.60	4.91	5.17	4.69	4.06	4.24	4.03	4.01					3.56 <sup>3</sup>
Mar. 31 U	26	250	2.38	2.92	3.85	4.18	3.03	2.27	2.74	2.48	2.35					1.96 <sup>3</sup>
Mar. 19 G	26	250	3.48	3.09	4.45	4.72	4.69	3.71	3.06	2.22	2.68					2.71
May 14 G	26	250	2.47	2.90	4.19	3.82	3.62	3.29	2.61	2.51	2.38					2.19 <sup>3</sup>
		Average	3.05	3.38	4.35	4.47	4.01	3.33	3.16	2.81	2.86					2.61
Feb. 12 U	35.8	350	3.08	3.08	3.95	4.60	5.48	4.03	3.60	3.66	4.18					2.78 <sup>3</sup>
Dec. 10 G	52	500	2.08	3.06	4.62	5.25	5.12	4.08	4.18	4.42	2.75 <sup>3</sup>					
Dec. 15 G	104	500	2.13	3.33	5.31	5.20	5.01	4.85	6.16	5.58						

<sup>1</sup> U=Universal apparatus; G=Gasometer apparatus.<sup>2</sup> Calculated to one half hour from 5 ten-minute periods.<sup>3</sup> Average of less than a half-hour (15 to 28 minutes).

last half of the third hour on. All of the values during the first three hours on March 5 show an increase in the carbohydrate metabolized. The maximum average summation of increases was 1.9 grams, equivalent to 19 per cent of the dextrose ingested. The water alone had but little effect upon the carbohydrate metabolized.

Four experiments with 15 grams of dextrose resulted in an increase in the respiratory quotient for the first three hours in all but three of the periods. The maximum increase was 0.06 in the third half hour on March 26. Many of the other increases were as high as 0.04. There were increases in the carbohydrate metabolized during the first two hours and in two of the experiments, March 26 and March 10, the increase persisted throughout the third hour. The greatest increase in any one half hour occurs in the third on March 26, 1.47 grams. The maximum average increment was 3.7 grams, equivalent to 25 per cent of the dextrose given. The experiments with 150 cc. of water alone gave exceptional results in that the decrease in carbohydrate metabolized was larger than after 100 cc. of water. In three hours the average drop was 3.3 grams, so that the maximum net effect of 15 grams of dextrose would be an increase of 7.0 grams in the carbohydrate metabolized.

In the four experiments with 21 grams the increases in the respiratory quotient were more marked and there was a positive rise in the first three hours in every case, the maximum being 0.07 on May 3 and March 15. In these experiments the increases in carbohydrate metabolized were also more marked than in any of the preceding groups. All experiments gave increases in each period for the first three half hours; the increase continued in three of the experiments through the eighth half hour, that is, over a total of four hours. There was a fair degree of uniformity in the four experiments in the increase in carbohydrate metabolized for the first four hours, 6.1 to 6.6 grams, after the ingestion of 21 grams; this increase is equal to about 30 per cent of the carbohydrate ingested. There was no increase in carbohydrate metabolized due to 200 cc. of water alone, but rather a fall on the average of 3.9 grams in four hours. If this is added to the change due to 21 grams of dextrose, it would raise the results so that the average net change in 4 hours would be +10.2 grams. However, the basal carbohydrate metabolized averaged 3.41 grams per half hour in the water experiments and 2.79 grams in the experiments with dextrose. The correction for the change due to the ingestion of the water would therefore probably not be 3.9 grams but less.

In the experiments with 26 grams there was a positive rise in the respiratory quotient for the first three hours in all but four instances. In general,

the rise in this group was not so high as with 21 grams. In two cases, however, the basal respiratory quotient was high, namely, January 25, 0.89, and March 19, 0.86. In the four experiments with 26 grams, there was increased metabolism of carbohydrate in practically all periods during the first three and one-half hours. The exceptions are the fifth half hour on March 31, and the first, sixth and seventh half hours on March 19. The maximum increase in any one half hour was on March 31, the third half hour, 1.8 grams. In general the greatest increases came in the second and third half hours. In the experiment on January 25, which has a high basal carbohydrate per half hour, 3.87 grams, the increases were continued throughout four hours, whereas with the slightly lower basal carbohydrate on March 19, there is a continuous decrease per half hour beginning with the sixth half hour. In general this group did not show so large an increase in the carbohydrate metabolized as the experiments with 21 grams, although the amount ingested here was 5 grams greater. The average increment in three hours was 4.4 grams, equivalent to 17 per cent of the sugar ingested. In this group the average basal metabolism of carbohydrate was 3.05 grams, whereas in the 21-gram group it was 2.79 grams, which may account in part for the lower increase with 25 grams. The increase in carbohydrate metabolized due to 250 cc. of water alone plays little or no rôle in the change due to ingestion of 25 grams of dextrose.

With 36 grams on February 12, in the fourth half hour, there was a rise of 0.10 in the respiratory quotient, and a rise in the quotient in all of the half hours with the exception of the first and ninth. There was an increase in the carbohydrate metabolized per half hour beginning with the second half hour after ingestion and continuing through the eighth. The total increase was 7.94 grams in four hours, thus equivalent to about one-fifth of the amount ingested. There was no experiment with 350 cc. of water alone. The experiments with 250 cc. gave an increase of 0.2 gram of carbohydrate in the first hour, and 500 cc. of water alone in one experiment resulted in an increase of 3.2 grams in 2 hours, so that about 1.5 grams of the rise after ingestion of 36 grams of dextrose may be ascribed to the water alone.

With 52 and 104 grams, there was a rise in the respiratory quotient of 0.10 in the second hour and a rise in all of the periods. The ingestion of 52 grams in 500 cc. of water produced increases in the carbohydrate metabolized in all the periods, the maximum occurring in the third half hour, 3.17 grams. During the four hours there was an increase of 16.8 grams in carbohydrate metabolized, which is equivalent to 32 per cent of the amount ingested. In the experiment with 104 grams, the increment was

20.5 grams in three and one-half hours, equivalent to 20 per cent of the dextrose ingested, but in neither of these experiments was the total increase in the metabolism of carbohydrate measured, as the amount per half hour did not fall to the basal level. The 500 cc. of water alone resulted in an increase of 3.2 grams in the carbohydrate metabolized, so that part of the rise due to 52 and 104 grams may be ascribed to the water of dilution.

The results show that, in general, quantities from 10 grams upwards of dextrose produce a positive rise in the respiratory quotient, beginning usually during the second half hour after ingestion, and the maximum quotient is obtained in the third half hour after ingestion. The average maximum rise in a single period for each group is shown in Figure 1.

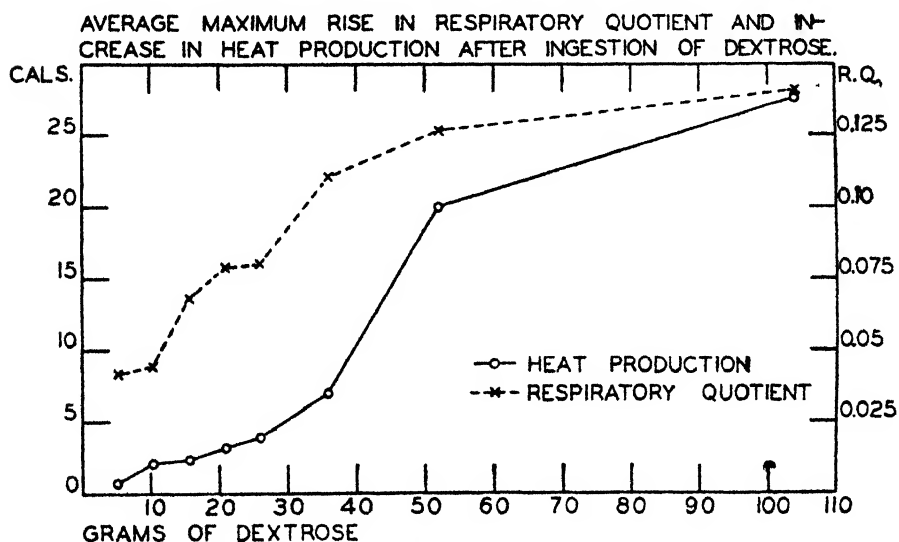


FIGURE 1. The upper line shows the average maximum increase in any single period for the respiratory quotient after the ingestion of dextrose. The lower line shows the average summation of increase in heat production above basal for each group.

It is evident that dextrose given in 10 gram amounts or over will raise the carbohydrate levels for a period of 1 hour or more, so that in order to supply carbohydrate equal to that metabolized by our subject in the basal condition dextrose should be given in amounts under 10 grams at intervals of one and one-half hours.

#### LENGTH OF TIME IN WHICH DEXTROSE INGESTED WOULD REPLACE THE CARBOHYDRATE METABOLIZED

The length of time in which the dextrose taken would replace the carbohydrate burned after the sugar is taken is given in Table III. In the

four experiments with 5.05 to 5.25 grams, the sugar ingested would last about an hour. The longest period is with the lowest basal carbohydrate of 1.68 grams on February 24, whereas the shortest period is with the highest basal carbohydrate of 3.17 grams on May 26. In the four experiments

TABLE III  
LENGTH OF TIME DURING WHICH INGESTED DEXTROSE WOULD REPLACE THE  
CARBOHYDRATE METABOLIZED

Date	Basal carbohydrate per half hour	Dextrose ingested	Replaces sugar metabolized
1926	grams	grams	minutes
May 26	3.17	5.05	45
June 2	2.39	5.05	59
Feb. 17	2.44	5.25	68
Feb. 24	1.68	5.25	74
Average	2.42		62
Apr. 26	3.99	10.1	71
Feb. 19	2.19	10.5	129
Mar. 1	2.76	10.5	91
Mar. 5	2.43	10.5	100
Average	2.84		98
Mar. 26	3.01	15.5	121
Feb. 26	3.56	15.75	107
Apr. 23	1.79	15.5	213
Mar. 10	2.70	15.75	129
Average	2.77		143
Mar. 3	2.91	21	158
Apr. 15	2.71	21	171
May 3	2.40	20	174
Mar. 15	3.13	21	149
Average	2.79		163
Jan. 25	3.87	25	161
Mar. 31	2.38	26	267
Mar. 19	3.48	26	211
May 14	2.47	26	249
Average	3.05		222

with 10.1 to 10.5 grams, the basal carbohydrate is higher on the average, and the sugar ingested would last about one and one-half hours. The inverse relationship between the basal carbohydrate and the length of time in which the dextrose would replace the carbohydrate burned holds in the 10-gram series. In the experiments with 15.5 and 15.75 grams, the average duration as compared with the 10-gram group is increased by 45 minutes, which corresponds nearly to the difference in duration between the 5 and the 10 gram groups, *i.e.*, 36 minutes. The additional duration of carbohydrate metabolized, supplied by the dextrose when 20 to 21 grams are taken, is 20 minutes. The group of 25-26 grams would supply the carbohydrate metabolized for 222 minutes, an increase of 59 minutes due to the extra 5 grams. With the higher amounts of 36, 52, and 104 grams, the metabolism of carbohydrate is relatively higher per minute, so that the dextrose ingested would not last so long. Calculated on a per gram basis the dextrose ingested would last 12, 9.4, 9.2, 8.0, and 8.5 minutes per gram of dextrose in the groups from 5 to 26 grams. This suggests that the smaller the quantity of dextrose the more economically it would be used, provided there was no need to raise the general carbohydrate level.

#### HEAT PRODUCTION AND SPECIFIC DYNAMIC ACTION<sup>1</sup>

The heat production per half hour in the basal period and in the half hours succeeding the ingestion of dextrose is given in Table IV. The basal heat production varied from 29.2 calories on April 26 and March 26 to 32.0 calories on April 15 and May 14.

Three of the four experiments with 5 grams of dextrose gave slightly lower values after the ingestion of dextrose. The fall in heat production was not quite so much as after 50 cc. of water alone, but the difference is not significant.

In the experiments with 10 grams, there were increases in the first hour in all four experiments, and on February 19, slight increases occurred successively through the fifth half hour. The maximum increase in any one half hour was 2.4 calories in the second on February 19. The experiments of February 19 and March 5 showed the greatest total increase, the maxi-

<sup>1</sup> Benedict and Carpenter (1918 p. 335) designated the relationship between the increase in heat production and the fuel value of the ingested material as the "cost of digestion." The expression "specific dynamic action" has also been used by some writers in the same sense, and as this phrase is the more commonly used as a synonym for the stimulating effect of food components upon total metabolism, the percentage relationship between the fuel value of the ingested sugar and the increase in heat production will be called "specific dynamic action" in this and the following article.



TABLE IV  
SUMMARY OF HEAT PRODUCED AFTER INGESTION OF DEXTROSE (Calories per half hour)

Date and apparatus <sup>1</sup>	Amount		Basal <sup>2</sup>	Half hours after ingestion of dextrose								
	Dextrose	Water		1	2	3	4	5	6	7	8	9
1926 May 26 U June 2 U Feb. 17 G Feb. 24 G	grams	cc.		30.4	30.4	29.6	28.4	29.9	30.1	29.3	30.8	
	5.05	50	30.5	31.0	30.3	29.7	30.0	30.3	30.2	30.2	31.2	31.5
	5.25	50	31.7	32.8	31.5	30.4	31.0	30.7	31.1	31.3	31.2	30.5
	5.25	50	30.0	30.9	31.6	30.1	30.4	30.0	30.5	30.3	30.4	
		Average	30.8	31.3	31.0	30.0	30.0	30.2	30.5	30.3	30.8	
Apr. 26 U Feb. 19 U Mar. 1 G Mar. 5 G	10.1	100	29.2	29.6	29.5	28.7	28.5	28.9	29.0	29.7	29.3	
	10.5	100	31.0	32.3	33.4	32.1	31.8	31.2	30.9	31.2	31.1	30.4
	10.5	100	30.7	31.1	31.1	29.0	29.2	29.0	29.4	29.9	30.2	30.2
	10.5	100	29.5	30.7	31.2	29.9	29.2	29.9	30.1	29.3	29.0	29.1
		Average	30.1	31.0	31.3	29.9	29.7	29.8	29.9	30.0	29.9	29.9
Mar. 26 U Feb. 26 U Apr. 23 G Mar. 10 G	15.5	150	29.2	29.7	30.3	29.7	28.4	28.7	28.8	28.3	29.2	29.8
	15.75	150	30.3	30.6	31.4	30.5	29.4	29.3	29.3	29.0	29.4	29.3
	15.5	150	31.6	32.7	32.0	29.6	30.0	30.2	30.8	31.2	31.9	
	15.75	150	30.1	31.8	33.1	31.1	29.7	29.7	29.8	30.5	31.1	31.3
		Average	30.3	31.2	31.7	30.2	29.4	29.5	29.7	29.8	30.4	30.1
Mar. 3 U Apr. 15 U May 3 G Mar. 15 G	21	200	30.0	31.1	32.0	30.4	29.2	29.5	29.1	29.3	29.4	29.6
	21	200	32.0	33.3	33.9	31.8	30.6	29.9	29.8	30.6	30.4	31.3
	20	200	31.3	33.5	32.8	30.3	31.1	31.1	31.2	32.0	32.6	
	21	200	30.4	31.9	31.8	30.1	29.5	29.7	29.9	30.2	30.7	31.0
		Average	30.9	32.5	32.6	30.7	30.1	30.1	30.0	30.5	30.8	30.6
Jan. 25 U Mar. 31 U Mar. 19 G May 14 G	25	250	29.7	31.6	32.1	31.2	29.8	29.6	29.5	29.5	29.3	30.3 <sup>a</sup>
	26	250	30.8	31.9	32.4	30.4	29.3	29.6	28.5	29.7	30.5	30.8 <sup>a</sup>
	26	250	30.0	31.8	32.2	31.3	31.2	30.2	29.8	29.3	30.0	30.3
	26	250	32.0	34.1	33.4	30.9	31.0	30.0	31.2	30.2	31.0	31.2 <sup>a</sup>
		Average	30.6	32.4	32.5	31.0	30.3	29.9	29.8	29.7	30.2	30.7 <sup>a</sup>
Feb. 12 U Dec. 10 G Dec. 15 G	35.8	350	29.7	31.4	33.1	31.6	29.7	29.5	29.2	29.6	30.4	30.6 <sup>a</sup>
	52	500	30.0	33.5	33.3	33.0	33.5	31.3	30.6	32.1	32.7 <sup>a</sup>	
	104	500	30.5	36.0	36.0	34.0	34.2	33.3	34.1	33.5		

<sup>1</sup> U=Universal apparatus; G=Gasometer apparatus.<sup>2</sup> Calculated to one-half hour from 5 ten-minute periods.<sup>a</sup> Average of less than a half-hour (15 to 28 minutes).

imum being 6 calories on February 19, and 4 calories on March 5. These increases are equivalent to 16 and 11 per cent, respectively, of the heat value of the dextrose ingested, *i.e.*, specific dynamic action, but if the average increase during the first hour is used (2.1 calories), there was a 6 per cent specific dynamic action. With 100 cc. of water<sup>1</sup> alone the change was +0.5 calorie the first hour, and -0.5 calorie the second hour. The correction for water alone would affect but little the maximum increase after 10 grams of dextrose.

With 15.5 to 15.75 grams of dextrose, there was an increase in the heat production in all four experiments during the first two half hours. The maximum summation of increments by half hours was 5.7 calories on March 10, and the average increase in the first hour was 2.3 calories, equivalent to 4 per cent of the heat value of the ingested dextrose. The results of the experiments with 150 cc. of water alone were anomalous in that they showed greater drop in heat production than any other group, so that correcting the above values for the drop after water alone would raise materially the changes in heat production.

In four experiments with 20 and 21 grams, the increases occurred only during the first hour. After this, for the most part, the values were lower than those for basal. One experiment gave an increase of 4.2 calories in four hours, and maximum summation of increments occurred only in the first hour in all of them, averaging 3.2 calories, which is equivalent to 5 per cent as the specific dynamic action. The effect of 200 cc. of water was to increase the heat production by 2.6 calories in 4 hours. This would nearly wipe out any effect due to dextrose alone, and therefore would bring about the result that 21 grams of dextrose were not so effective in increasing the heat production as smaller amounts.

In four experiments with 25 and 26 grams, there was an increase in heat production during the first two hours on January 25 and March 19. On May 14 and March 31, the increase occurred mainly during the first hour. In general, the heat production was slightly greater after the ingestion of 25 grams than after 21 grams. The maximum summation of increases was 6.5 calories in two hours on March 19, and the average increase in two hours was 3.7 calories, equivalent to 4 per cent of the fuel value of the dextrose ingested. The heat production after 250 cc. of water alone was increased 1.2 calories the first hour, which would leave 2.4 calories as the increment in one hour due to 26 grams of dextrose alone.

The heat production in the experiment with 35.8 grams rose markedly in the first three half hours. The maximum increase was 3.4 calories in

<sup>1</sup> For the effect upon metabolism of ingestion of water alone, see previous paper, p. 359.

the second half hour. The greatest increase summed to 7.0 calories in 2 hours, *i.e.*, 5 per cent specific dynamic action. No experiments were made with 350 cc. of water alone, but one may interpolate the results between 250 and 500 cc. This would give as the increase in heat production during the first hour due to water alone 1.8 calories, which would leave 3.3 calories due to the dextrose alone. It is evident that some of the increase in heat production after 350 cc. of 10 per cent solution of dextrose may be due to water alone.

With 52 grams, the maximum increase was 3.5 calories in the first and fourth half hours, and the minimum increase was 0.6 calorie in the sixth half hour. Throughout the first two hours there was an increase of 10 per cent or over. The increase in four hours was 20.0 calories, and this apparently does not include the total increase due to 52 grams of dextrose, as the heat production had not reached basal at the end of four hours. The difference between 36 grams and 52 grams is 16, and this additional amount caused a greater additional increase in heat production than any quantity under 36 grams.

With 104 grams, there was an increase of 5.5 calories in each of the first two half hours, *i.e.*, 18 per cent above the basal heat production. The experiment was only three and one half hours in length, and during this time there was an increase of 27.6 calories, *i.e.*, 13 per cent above basal, but this does not include all the heat increase due to 104 grams, as the heat production had not reached basal during the 3 1/2 hours.

With 52 grams, the increase of 20 calories equals 10.3 per cent as the specific dynamic action and with 104 grams, the increase of 27.6 calories in three and a half hours equals 7.1 per cent, but in neither of these was the duration long enough to measure all of the heat increment. Benedict and Carpenter (1918) found with 75 and 100 grams of dextrose from 2 to 9 per cent, and Deuel (1927) found 8 per cent with 75 grams of glucose.

Only one experiment with 500 cc. of water alone was made, and this resulted in an increase of 8.0 calories in 2 hours. The subtraction of this amount from the increases of 13.3 and 18.2 calories after 52 and 104 grams would lower materially the increase due to dextrose alone. It is evident that further investigation is necessary to determine whether there is a real difference in heat production as affected by sugars given dry or given in 10 per cent solution. This situation raises the question as to how much of the effect of food is due specifically to the food itself and how much to the fluids taken along with it.

Apparently there is a critical level in the relationship between the energy value of dextrose and the increment of heat production after the ingestion

of a 10 per cent solution of this sugar. This level occurs with amounts of dextrose up to and including 25 grams; with these amounts the increment in heat production equals about 4 per cent of the energy value of the dextrose. Above 25 grams the increase in heat production is greater. The average summated increase in heat production for each group is shown in Figure 1, page 381.

This finding is of value where it is desirable to give dextrose without raising noticeably the heat production. It is evident that 25 grams or under may be given at intervals of one and a half hours without causing a rise in the heat production of over 6 per cent. Such a procedure would be useful when one wishes to supply carbohydrate just sufficient to be above the level of ketogenesis, but does not want to produce glycosuria. Amounts above 25 grams would lead to greater increases in heat production which in turn would require a greater energy supply for maintenance. It should be noted that 25 grams far exceeds the maximum requirements for carbohydrate supply for one and a half hours. The greatest carbohydrate use in the first three half hours after dextrose ingestion in these experiments was 14.7 grams on January 25 when 25 grams were taken. An amount between 10 and 15 grams would be equivalent to the sugar used in one and a half hours with this subject. The amount to be taken would also depend on how low a carbohydrate level the subject had, when the ingestion began.

#### SUMMARY

The respiratory exchange of a human in the post-absorptive condition was determined by two different methods, for from three to four hours after the ingestion of dextrose in amounts between 5 and 104 grams in water at 37° C.

In the majority of the observations, the maximum respiratory quotient was observed in the third half hour, and all amounts of 10 grams or over were accompanied by significant changes in the respiratory quotient.

Increases in the metabolism of carbohydrate were observed in the first one and one-half hours with amounts of 5 to 10 grams, in 3 hours with 15 grams and in three and one-half to four hours with 21 to 25 grams.

The increase in carbohydrate metabolism above basal for 3 hours represented on the average from 17 to 31 per cent of the amount of dextrose ingested in quantities of 10 to 36 grams.

The increase in heat production was practically 1 calorie for each additional 5 grams from 5 to 25 grams. The increase in the heat production above 25-gram amounts rose more rapidly in proportion to the additional amounts given.

The specific dynamic action, that is, the relation of the increase in heat production to the energy value of the ingested dextrose, varied from 4 to 6 per cent when 5 to 36 grams were given. When 52 grams were taken, the value was 10 per cent.

#### BIBLIOGRAPHY

- Benedict, F. G., and Carpenter, T. M., 1918, *Carnegie Institution of Washington, Pub. No. 261*, 338.  
Carpenter, T. M., 1925, *Carnegie Institution of Washington Pub.* 369, 158.  
Deuel, H. J., Jr., 1927, *Jour. Biol. Chem.*, LXXV, 388.  
Lambie, C. G., and Redhead, F. A., 1929, *Biochem. Jour.*, XXIII, 610.  
Lublin, A., 1926, *Arch. f. exper. Path. u. Pharmacol.*, CXV, 101.  
Mason, E. H., 1926, *Jour. Clin. Invest.*, II, 521.



# THE GASEOUS EXCHANGE OF THE HUMAN SUBJECT

## III. AS AFFECTED BY SMALL QUANTITIES OF LEVULOSE

BY THORNE M. CARPENTER AND EDWARD L. FOX

*(From the Nutrition Laboratory of the Carnegie Institution of  
Washington, Boston, Mass.)*

Received for Publication—September 24, 1929

IN A PREVIOUS investigation in this Laboratory (Carpenter 1925), an experiment with 30 grams of levulose in 500 cc. of water was reported in which the respiratory exchange was measured. There was a marked rise in the respiratory quotient, which was nearly as large as that observed in an earlier study (Benedict and Carpenter 1918) with the much larger quantities of 75 and 100 grams. This result indicated the desirability of experiments with small quantities of levulose in order to determine the minimum quantity that would produce a measurable change in the respiratory quotient, and in the metabolism as represented by the calculated heat production. Also, in view of the characteristic course of the respiratory quotient after comparatively large amounts of levulose as compared with that after dextrose, the question arose as to whether this typical curve would occur when the quantities were much smaller.

The number of experiments in which levulose has been given to man in quantities under 50 grams, and the respiratory exchange measured, is relatively small. Lublin (1926) reported two experiments with 20 grams and three experiments with 30 grams of levulose. The respiratory quotient rose markedly within an hour in all of the experiments and there was also an increase in the consumption of oxygen.

The methods of measuring the respiratory exchange before and after the ingestion of levulose were the same in the experiments here reported as in the preceding studies on the effect of the ingestion of water and of small quantities of dextrose. (*This Jour.* 1930, II, 359 and 375.) The levulose used was Kahlbaum's commercial grade of levulose, which was weighed out as needed, dissolved in pure water and warmed (in all but one experiment) to 37°C. The solution was usually taken by sucking it through a small rubber tube and using 25 cc. of water for final rinsing of the container and tubing.

### RESULTS OF INGESTION OF LEVULOSE

Basal values and results in half hours following the ingestion of the levulose are given for the respiratory quotient, carbohydrate metabolized

and heat produced in all the experiments with the gasometer method and the Universal apparatus. As in the preceding two papers, the first of the basal periods was rejected and the basal values given were derived from the average of periods 2 to 6 inclusive, in the basal hour preceding the ingestion of sugar.

As the respiratory quotient is computed from the total respiratory exchange, the metabolism of protein must be obtained for the calculation of the total heat produced and consequently for the carbohydrate metabolized. These computations have been carried out for the basal period and for the half hours after the ingestion of levulose in the same manner as in the experiments with water and with dextrose, the average urinary nitrogen per hour in the experiments with levulose being 0.43 gram for the experiments with the Universal apparatus and 0.39 gram for the experiments with the gasometer apparatus.

#### RESPIRATORY QUOTIENT AND METABOLISM OF CARBOHYDRATE

The respiratory quotients and carbohydrate metabolized, are given for the experiments with levulose in Tables I and II. In general, the ingestion of 5 grams of levulose was followed by a slight rise in the respiratory quotient within an hour. The metabolism of carbohydrate was definitely increased in the first half hour of five of the experiments with 5 grams of levulose. On April 16 and February 5 this increase continued to the fourth half hour, and on the latter date through the ninth half hour. In the experiment on June 14, there was an increase lasting for one hour. The average increase in carbohydrate metabolized in the first half hour after 5 grams of levulose was 0.4, and in the second half hour, 0.3 gram.

On the days when 10 grams of levulose were ingested, there was a definite rise in the respiratory quotient in the second half hour in all the experiments and in the first half hour in three experiments. In general, the increase in quotient in the second half hour of the four experiments with this amount of levulose was greater than the increase after 5 grams of levulose. The carbohydrate metabolized was increased in all of the four experiments. The average increase in half hours 1 to 4 was 0.9, 1.7, 0.4, and 0.2 gram, respectively. The average summation<sup>1</sup> of increases for 2 hours in the four experiments was 3.1 grams.

In the experiments with 15.5 grams of levulose, there was an increase in

<sup>1</sup> By this expression is meant the greatest increase that can be obtained mathematically by adding the increases in successive half hours. The number of half hours so used will vary in the different experiments according to the duration of increases as well as the amount of levulose ingested.

the respiratory quotient lasting on January 8 throughout the  $4\frac{1}{2}$  hours of measurement, on March 22 for 3 hours, and on January 27 and March 29 for  $1\frac{1}{2}$  hours. There was thus a difference between the results obtained with the Universal apparatus on January 8 and March 22 and those obtained with the gasometer apparatus on January 27 and March 29. The increase in respiratory quotient during  $1\frac{1}{2}$  hours in these experiments with 15.5 grams was greater than that after either 5 or 10 grams of levulose. The metabolism of carbohydrate was increased immediately following the ingestion of levulose in all of the four experiments, but this increase varied in duration in the several experiments, lasting from  $1\frac{1}{2}$  hours to  $4\frac{1}{2}$  hours. The greatest increases occurred in the second half hour, in which the maximum was 4.06 grams on March 29. The average summation of increases for 3 hours in the four experiments was 8.2 grams.

Following the ingestion of 21 grams of levulose, there was a marked increase in respiratory quotient in the second half hour in all four experiments, and in the first half hour, in three of the experiments. The increases in the quotients lasted from 2 to 3 hours and were, in general, somewhat more sustained than in the preceding groups with levulose. The metabolism of carbohydrate was increased in all cases for at least 2 hours; on March 17,  $2\frac{1}{2}$  hours and on January 2 and November 27, 3 hours. The average summation of increases for 3 hours in the four experiments was 7.2 grams. Compared with the increases for the 15.5-gram group, the increases after 21 grams of levulose were mostly smaller, both by successive half hours and as summation of increases. The average basal carbohydrate was somewhat lower in the 15.5-gram group than in the 21-gram group, *i.e.*, 2.30 as compared with 3.06 grams. This fact may account for the higher increases after 15.5 grams of levulose.

In all of the six experiments with 26 grams of levulose, there was a definite increase in the respiratory quotient in the first half hour, and in five of the experiments these increases lasted from 2 to  $3\frac{1}{2}$  hours. The quotients were more sharply increased in the first hour than in the same length of time in the 21-gram group. There were increases in carbohydrate metabolized for from 2 to  $3\frac{1}{2}$  hours in these experiments with 26 grams, the maximum increase in any half hour being 3.8 grams in the second half hour on November 19. The average summation of increases for 3 hours in the six experiments was 7.8 grams. These changes in carbohydrate metabolized are of the same order of magnitude as those noted after the ingestion of 21 grams of levulose.

In one of the two experiments with 52 grams, the rise in respiratory quotient was 0.17 in the second half hour, which was equalled in the



TABLE I  
SUMMARY OF RESPIRATORY QUOTIENTS AFTER INGESTION OF LEVULOSE

Date and apparatus <sup>1</sup>	Amount ingested		Basal <sup>2</sup>	Half hours after ingestion of levulose.										
	Levulose	Water		1	2	3	4	5	6	7	8	9		
1926 Jan. 18 U Jan. 22 U Feb. 5 G Apr. 16 G June 9 G June 14 G	grams	cc.	0.89	0.91	0.87	0.83	0.85	0.85	0.86	0.85	0.86	0.85	0.84	0.83
	5.05	50	.88	.91	.88	.87	.87	.86	.87	.87	.87	.87	.84	.84 <sup>2</sup>
	5.05	50	.79	.82	.81	.82	.81	.79	.82	.81	.81	.81	.81	.81
	5.05	50	.83	.83	.86	.85	.84	.81	.81	.81	.81	.81	.81	.81
	5.05	50	.84	.81	.84	.81	.79	.80	.81	.81	.81	.81	.80	.80
	5.05	100	.81	.86	.83	.80	.81	.83	.83	.83	.80	.80 <sup>2</sup>		
Jan. 13 U Apr. 21 U Feb. 1 G Apr. 12 G		Average	.84	.86	.85	.83	.83	.82	.82	.82	.83	.83	.82	.82
	10.1	100	.88	.89	.96	.90	.88	.89	.87	.85	.87	.86	.86	.83
	10.1	100	.82	.83	.86	.83	.82	.82	.83	.82	.83	.83	.81	.81
	10.1	100	.83	.91	.89	.84	.85	.84	.83	.81	.81	.82	.82	.83
	10.1	100	.80	.77	.87	.83	.81	.80	.81	.79	.80	.80	.80	.80
		Average	.83	.85	.90	.85	.84	.84	.84	.84	.82	.84	.82	.82
Jan. 8 U Mar. 22 U Jan. 27 G Mar. 29 G		15.5	.81	.89	.95	.86	.87	.86	.86	.86	.87	.84	.83	.85 <sup>2</sup>
	15.5	150	.81	.84	.94	.88	.86	.85	.85	.84	.84	.81	.80	.82 <sup>2</sup>
	15.5	150	.85	.92	.96	.87	.83	.80	.81	.81	.81	.81	.81	.82
	15.5	150	.80	.86	.95	.86	.81	.81	.81	.81	.81	.80	.80	.81
		Average	.82	.88	.95	.87	.84	.83	.83	.83	.83	.83	.81	.82
	21	200	.83	.87	.95	.91	.89	.85	.85	.86	.86	.82	.84	.85
Jan. 2 U Mar. 17 U Nov. 27 <sup>4</sup> G Dec. 4 <sup>4</sup> G		21	.83	.85	.93	.92	.88	.89	.89	.83	.83	.82	.81	.81 <sup>2</sup>
	21	200	.84	.83	.90	.92	.87	.86	.86	.86	.86	.84	.85	.84
	21	200	.87	.92	.96	.91	.89	.86	.86	.86	.86	.82	.83	.83
	21	200	.84	.87	.94	.92	.88	.87	.86	.86	.86	.82	.83	.83
		Average	.84	.87	.94	.92	.88	.87	.87	.87	.85	.83	.83	.83

TABLE I. CONT'D

Dec. 28 <sup>1</sup> U	26	250	.94	1.02	1.00	.90	.93	.94	.90	.92	.89	.89
Mar. 13 U	26	250	.83	.89	.98	.92	.90	.86	.87	.86	.83	.83 <sup>2</sup>
Nov. 19 <sup>4</sup> G	26	250 <sup>5</sup>	.81	.85	.94	.91	.91	.83	.81	.82	.79	.80
Dec. 17 <sup>4</sup> G	26	250	.85	.88	1.02	.94	.89	.86	.85	.83	.84	.85
Mar. 24 G	26	250	.80	.83	.93	.89	.83	.84	.82	.79	.79	.81
Sept. 23 G	26	250	.88	.92	1.00	.98	.90	.87	.86	.84	.83 <sup>2</sup>	
		Average	.85	.90	.98	.92	.89	.87	.85	.84	.83	.84
Dec. 8 G	52	500	.80	.92	.88	.88	.88	.84	.83	.88 <sup>3</sup>		
Dec. 22 G	52	500	.81	.89	.98	.90	.87	.87	.86	.81		
		Average	.81	.91	.93	.89	.88	.86	.85	.85		
Dec. 13 G	104	500	.84	.98	1.01	.98	1.00	.96	.92	.89	.92 <sup>2</sup>	

<sup>1</sup> U = Universal apparatus; G = Gasometer apparatus.<sup>2</sup> Average respiratory quotient for five 10-minute periods.<sup>3</sup> Respiratory quotient for less than a half hour (15 to 29 minutes).<sup>4</sup> 1925.<sup>5</sup> The water in the experiment of Nov. 19, 1925 was at 16.8°C.

TABLE II  
SUMMARY OF CARBOHYDRATE METABOLIZED AFTER INGESTION OF LEVULOSE (Grams per half hour).

Date and apparatus <sup>1</sup>	Amount ingested		Basal <sup>2</sup>	Half hours after ingestion of levulose								
	Levulose	Water		1	2	3	4	5	6	7	8	9
	grams	cc.										
1926												
Jan. 18 U	5.05	50	4.24	4.75	3.50	2.63	2.97	3.06	3.21	3.03	2.89	2.65
Jan. 22 U	5.05	50	3.88	4.50	3.87	3.69	3.44	3.37	3.46	3.44	2.60	2.77 <sup>a</sup>
Feb. 5 G	5.05	50	1.54	2.20	2.65	2.34	2.05	1.65	2.32	2.08	1.88	1.88
Apr. 16 G	5.05	50	2.55	2.68	3.58	3.28	2.76	2.13	2.16	2.14	2.14	2.20
June 9 G	5.05	50	2.96	2.33	3.03	2.28	1.83	2.05	2.24	2.20	1.79	
June 14 G	5.05	100	2.26	3.46	2.58	1.91	2.15	2.82	2.58	1.97	1.98 <sup>a</sup>	
	Average		2.91	3.32	3.20	2.69	2.53	2.51	2.66	2.48	2.21	2.38
Jan. 13 U	10.1	100	3.69	3.86	5.72	4.42	3.84	3.74	3.58	2.97	3.19	2.68
Apr. 21 U	10.1	100	2.43	2.92	3.54	2.56	2.38	2.59	2.82	2.31	2.14	2.12
Feb. 1 G	10.1	100	2.56	4.99	4.35	2.67	2.96	2.94	2.76	2.08	2.27	2.70
Apr. 12 G	10.1	100	2.03	2.36	3.87	2.59	2.22	1.77	2.18	1.59	1.85	1.77
	Average		2.68	3.53	4.37	3.06	2.85	2.76	2.84	2.24	2.36	2.32
Jan. 8 U	15.5	150	2.08	4.25	5.97	3.46	3.40	3.21	3.33	2.74	2.57	2.88 <sup>a</sup>
Mar. 27 U	15.5	150	2.07	3.06	5.81	3.64	3.21	2.91	2.86	2.02	1.87	2.26 <sup>a</sup>
Jan. 27 G	15.5	150	3.13	5.75	6.83	3.97	3.06	1.91	2.38	2.84	2.31	2.54
Mar. 29 G	15.5	150	1.92	3.48	5.98	3.36	2.07	2.04	2.07	1.74	1.87	2.07
	Average		2.30	4.14	6.15	3.61	2.94	2.52	2.66	2.34	2.16	2.44
Jan. 2 U	21	200	2.68	4.06	6.21	5.19	4.54	3.26	3.62	2.42	3.01	3.24
Mar. 17 U	21	200	2.49	3.04	4.93	4.55	3.78	3.94	2.56	2.19	1.83	1.97 <sup>a</sup>
Nov. 27 <sup>a</sup> G	21	200	3.19	3.09	4.64	5.47	3.97	3.67	3.62	3.14	3.46	2.92
Dec. 4 <sup>a</sup> G	21	200	3.87	5.48	6.45	4.96	4.17	3.54	3.48 <sup>a</sup>	2.59	2.53	2.56
	Average		3.06	3.92	5.56	5.04	4.12	3.60	3.32	2.59	2.71	2.67

TABLE II. CONT'D

Dec. 28 <sup>a</sup> U	26	250	5.14	6.12	6.34	4.72	5.08	5.71	4.42	4.55	3.98	3.83
Mar. 13 U	26	250	2.62	4.13	6.01	4.81	4.27	3.37	3.60	3.19	2.62	2.42 <sup>b</sup>
Nov. 19 <sup>a</sup> G	26	250 <sup>c</sup>	2.14	3.01	5.94	4.74	4.66	2.70	2.15	2.39	1.76	1.97
Dec. 17 <sup>a</sup> G	26	250	3.06	3.99	6.59	5.60	4.17	3.39	3.14	2.52	2.71	3.02
Mar. 24 G	26	250	1.92	2.90	5.28	4.08	2.52	2.73	2.32	1.48	1.73	2.18
Sept. 23 G	26	250	3.71	5.14	6.48	6.37	4.55	3.66	3.35	2.84	2.68 <sup>b</sup>	
		Average	3.10	4.22	6.11	5.05	4.21	3.59	3.16	2.83	2.58	2.68
Dec. 8 G	52	500	2.13	5.70	4.52	4.49	4.19	3.28	2.85	4.34 <sup>b</sup>		
Dec. 22 G	52	500	2.21	5.05	7.16	5.09	3.95	4.05	3.73	2.11		
		Average	2.17	5.38	5.84	4.79	4.07	3.67	3.29	3.23		
Dec. 13 G	104	500	2.79	7.16	7.73	7.77	7.77	7.42	5.90	4.93	5.70 <sup>b</sup>	

<sup>1</sup> U = Universal apparatus; G = Gasometer apparatus<sup>2</sup> Calculated to one half hour from five 10-minute periods.<sup>3</sup> Calculated from results for less than a half-hour (15 to 20 minutes).<sup>4</sup> 1925.<sup>5</sup> The water in the experiment of Nov. 19, 1925 was at 16.8°C.

preceding groups only in the second half hour after 26 grams on December 17. The increase in the respiratory quotient and the carbohydrate metabolized lasted  $3\frac{1}{2}$  hours in one experiment and three hours in the other. The two experiments gave total increases in carbohydrate metabolized of 14.5 and 15.7 grams, respectively, in  $3\frac{1}{2}$  hours, which are twice as great as those after half as much levulose, but we cannot be certain that all of the increase in carbohydrate was measured in the experiments with 52 grams, as the experiments were continued only  $3\frac{1}{2}$  hours after the ingestion of levulose, and the carbohydrate metabolized was still above the basal amount in the last half hour on December 8.

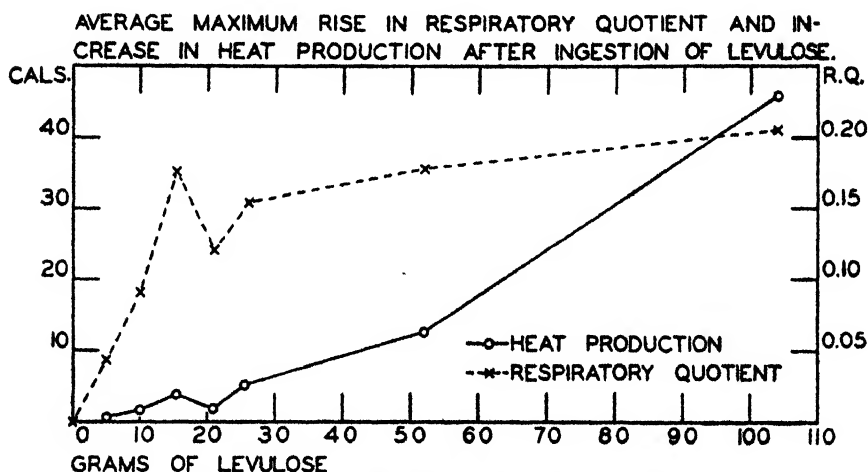


FIGURE 1. The upper line shows the average maximum increase in any single period for the respiratory quotient after the ingestion of levulose. The lower line shows the average summation of increase in heat production above basal for each group.

After the ingestion of 104 grams of levulose, there was a marked increase in the respiratory quotient which lasted at least the 4 hours of the observations. The carbohydrate metabolized did not return to the basal value in 4 hours. The total increase in 4 hours was 32.1 grams, which was twice as large as with one half the quantity of levulose, *i.e.*, 52 grams, but the experiment was not long enough to measure all the increase in carbohydrate.

The average of the maximum rise of the respiratory quotient in each experiment is shown for each group in Figure 1.

In general, there was a slight increase in carbohydrate metabolized after the ingestion of the smaller amounts of levulose, which rises with the amount ingested till the levulose is 15.5 grams. For 15.5 to 26 grams of

levulose, there is a plateau in the total increase of carbohydrate metabolized at about 7 to 8 grams, and above 26 grams, the increase in carbohydrate is at least proportional to the additional amounts given and may rise more rapidly than the increase in amount given.

#### RELATION OF INCREASE IN CARBOHYDRATE METABOLIZED TO LEVULOSE INGESTED

In the experiments with 5 grams of levulose there was an average summation of increases of 0.7 gram in carbohydrate metabolized, equivalent to 14 per cent of the ingested levulose. For 10 to 104 grams of levulose, the increase measured was equal to from 29 to 34 per cent of the levulose ingested, except for 15.5 grams of levulose which resulted in an increase in carbohydrate metabolized equivalent to 53 per cent. It is probable that in the last three experiments the total increase was not measured, so that the real percentages represented by the increases after 52 and 104 grams of levulose would be somewhat higher than those given above. In general, these percentages are somewhat higher than those found in the experiments with small quantities of dextrose.

#### LENGTH OF TIME IN WHICH LEVULOSE INGESTED WOULD REPLACE CARBOHYDRATE METABOLIZED.

The length of time in which the ingested levulose would replace the carbohydrate metabolized after the levulose was taken is given in Table III. In the six experiments with 5 grams of levulose there was a wide range in the basal carbohydrate and the extremes in duration of replacement bear inverse relationships to this range. Doubling the amount ingested to 10 grams increases, on the average, the duration of levulose as the supply by about 80 per cent. Again the maximum duration accompanies the lowest basal carbohydrate, and, *vice versa*, the minimum duration accompanies the highest carbohydrate. The addition of 5.4 grams of levulose, raising the amount ingested to 15.5 grams, increases the length of time the supply would last to 109 minutes on the average, *i.e.*, an increase of 23 minutes. The average for the 21-gram group is 31 minutes longer than that for the 15.5-gram group, an increase slightly greater than the difference in duration for the 10-gram and 15.5-gram groups. In the 26-gram group, the sugar would replace the carbohydrate metabolized for over three hours, or an additional 49 minutes over the average for the 21-gram group. The inverse relationship between high basal carbohydrate and shorter duration of the supply holds for the most part in the individual experiments. The larger amounts of 52 and 104 grams would replace the carbohydrate

TABLE III  
LENGTH OF TIME IN WHICH LEVULOSE INGESTED WOULD REPLACE CARBOHYDRATE METABOLIZED

Date	Basal carbohy- drate metabolized per half hour	Levulose given	Duration of replacement	Date	Basal carbohy- drate metabolized per half hour	Levulose given	Duration of replacement
1926				1926			
Jan. 18	grams 4.24	grams 5.05	mins. 33	Jan. 2	grams 2.68	grams 21	mins. 129
" 22	3.88	"	34	Mar. 17	2.49	"	159
Feb. 5	1.54	"	63	Nov. 27 <sup>1</sup>	3.19	"	151
April 16	2.55	"	50	Dec. 4 <sup>1</sup>	3.87	"	120
June 9	2.96	"	57				
June 14	2.26	"	48	Average	3.06		140
Average	2.91		48				
Jan. 13				Dec. 28	5.14	26	140
April 21	3.69	10.1	64	Mar. 13	2.62	"	178
Feb. 1	2.43	"	104	Nov. 19 <sup>1</sup>	2.14	"	217
April 12	2.56	"	69	Dec. 11 <sup>1</sup>	3.06	"	172
	2.03	"	107	Mar. 24	1.92	"	281
				Sept. 23	3.71	"	148
Average	2.68		86	Average	3.10		189
Jan. 8							
Mar. 22	2.08	15.5	106	Dec. 8	2.13	52	208 <sup>2</sup>
Jan. 27	2.07	"	118	Dec. 22	2.21	"	210 <sup>2</sup>
Mar. 29	3.13	"	82				
	1.92	"	129	Dec. 13	2.79	104	231 <sup>4</sup>
Average	2.30		109				

<sup>1</sup> 1925.    <sup>2</sup> 29.4 grams of carbohydrate.    <sup>3</sup> 31.1 grams of carbohydrate.

<sup>4</sup> 54.4 grams of carbohydrate.

metabolized for a somewhat longer period of time than the experiments lasted, as on the average only 30.3 grams of carbohydrate were metabolized in 209 minutes after the ingestion of 52 grams, and 54.4 grams of carbohydrate after the ingestion of 104 grams. Beginning with the 15.5 grams of levulose the increases in the duration of replacement of the carbohydrate burned are greater than would be expected from the additional amounts of levulose ingested.

### HEAT PRODUCTION

The values for the heat production in the basal periods and in the half hours after the ingestion of levulose, are given in Table IV. In five of the six experiments in the 5-gram group, there was an increase in the first half hour, and in four, there was an increase in the second half hour. Beginning with the third half hour, the changes were mostly in the minus direction. The average change in heat production during 1 hour was +0.5 calorie.

In three of the experiments with 10 grams there was an increase in heat production in the first half hour, and in all four an increase in the second half hour. The average increase in the first half hour in this group was 1.0 calorie; in the second, 0.9 calorie. The average fall in this group, from half hour to half hour, beginning with the third half hour, was not so large as in the group with 5 grams of levulose. The average increase in 2 hours was 1.1 calories in contrast to the decrease in 2 hours found with the 5-gram group.

There were positive increases in the first two half hours in all four experiments with 15.5 grams, which ranged from 1.0 to 3.6 calories. In the experiment on March 22, there was practically no change after the first hour. Although there was a high basal value on January 27, the heat production was increased in each succeeding half hour. The average increase for the four experiments amounted to 1.9 calories both in the first and in the second half hours, and there was a slight increase, 0.3 calorie on the average, in the third half hour. The average increase in 3 hours after 15.5 grams of levulose was 4.0 calories. The 15.5-gram group as a whole gave a greater increase in heat production than the two preceding groups.

Three out of four experiments with 21 grams, namely those with the high basal heat production, gave increases in the first and second half





TABLE IV CONT'D

Dec. 28 <sup>a</sup> U	26	250	29.9	31.6	32.5	31.9	30.6	31.6	30.2	29.8	30.0	30.2	30.7
Mar. 13 U	26	250	30.0	30.8	31.1	30.2	30.5	29.8	30.0	29.9	30.0	30.0	29.9 <sup>a</sup>
Nov. 19 <sup>a</sup> G	26	250 <sup>a</sup>	30.7	31.1	33.1	31.5	31.0	30.1	30.8	31.2	31.1	31.2	31.2
Dec. 17 <sup>a</sup> G	26	250	31.5	32.1	33.0	32.5	31.9	30.8	30.4	30.4	30.3	30.4	31.2
Mar. 24 G	26	250	30.5	32.0	32.0	31.3	30.4	30.4	30.4	30.0	30.7	30.0	31.1
Sept. 23 G	26	250	30.2	32.4	32.5	32.1	31.5	29.9	30.5	31.5	32.0 <sup>a</sup>	32.0 <sup>a</sup>	31.1
		Average	30.5	31.7	32.4	31.6	31.0	30.4	30.4	30.5	30.7	30.7	30.7
Dec. 8 G	52	500	33.2	35.4	34.1	33.9	33.4	33.4	33.7	34.4 <sup>a</sup>			
Dec. 22 G	52	500	31.5	35.9	35.4	34.7	33.3	34.1	33.4	33.1			
		Average	32.4	35.7	34.8	34.3	33.4	33.8	33.6	33.8			
Dec. 13 G	104	500	31.0	35.4	37.8	38.0	38.0	37.6	36.4	35.2	35.4 <sup>a</sup>		

<sup>a</sup> U = Universal apparatus; G = Gasometer apparatus.<sup>b</sup> Calculated to one half hour from five 10-minute periods.<sup>c</sup> Calculated from results for less than a half hour (15 to 29 minutes).<sup>d</sup> 1925.<sup>e</sup> The water in the experiment of Nov. 19, 1925 was at 16.8°C.

hours, and two of the three also gave increases in the third and fourth half hours. These three experiments, together with the one on January 27 in the 15-gram group, suggest that the reaction to the ingestion of levulose is greater when the basal metabolism is high than when it is low. The average increase in the first half hour for the four experiments was 0.7 and in the second, 0.5 calorie. These increases are not so great as in the two preceding groups with 10 and 15.5 grams of levulose and the average decreases in the succeeding half hours, namely from 0.5 in the fifth half hour to 1.2 calories in the ninth half hour, were greater than in those groups. For the group as a whole, therefore, there was not so marked an increase in metabolism as for the preceding group.

In all six experiments with 26 grams there was an increase in the heat production in each of the first three half hours, and in five of the experiments there was an increase in the fourth half hour. There was a greater general increase in the heat production in the first four half hours, and a smaller change in the next five half hours than in any of the preceding groups. The net increases were of about the same order as those for 15.5 grams and were not so great as one would expect if the increases were proportionate to the amount of levulose ingested. With the exception of the 21-gram group, there was progression in the increases in heat production as the amount of levulose ingested was increased.

In both experiments with 52 grams, all succeeding half hours after the ingestion of the levulose showed an increase, the maximum being 4.4 calories in the first half hour on December 22. The total increase in  $3\frac{1}{2}$  hours, which was as long as the experiments were continued, was 12.7 calories. Both in 1 hour and in 2 hours the increase in heat production after 52 grams was nearly twice as much as it was in corresponding periods with one half the quantity of levulose. In three hours the increase was two and one-half times as great as it was with one half the quantity of levulose.

In the one experiment with 104 grams, there were increases in all of the periods measured and, in all likelihood, the total increase in heat production was not obtained, as the increase evidently continued longer than 4 hours. The maximum increase of 7 calories occurred both in the third and in the fourth half hour, and was equivalent to 22.6 per cent of the basal value. The increase in 1 hour was 11.2 calories, which is nearly twice the increase in 1 hour after 52 grams of levulose. In 2 hours it was 25.2 calories, which is nearly three times the increase after 52 grams; in 3 hours it was 37.2 calories, which is over three times the increase after 52 grams; and in 4 hours it was 45.8 calories, which is about ten times as much as the

increase in 4 hours with 26 grams, that is, one-quarter of the amount of levulose given in this experiment.

There was a gradual ascent in the increase in heat production above the basal values when quantities of levulose from 5.05 to 15.5 grams were taken. The increase after 26 grams was about the same as after 15.5 grams, but the increase after 21 grams was not so large as after 10 and 15.5 grams. The calculation of the true effect should include the differences in the negative changes in the periods following those in which increases were found. These negative changes were not so large for the 10, 15.5, and 26-gram groups as for the 5 and 21-gram groups. The summation of the average negative changes in the fifth to ninth half hours for the 5 and 21-gram groups was 3.7 and 4.4 calories, respectively, whereas in the 10, 15 and 26-gram groups it was 1.9 calories, 0.7 and +0.3 calorie, respectively. There was, therefore, in addition to the positive increases in heat production, a difference in the various groups of nearly 4 calories in the downward course of the heat production in the periods following those in which there was a rise in heat production over basal. The real elevation in heat production for the 10, 15 and 26-gram groups was greater by 4 calories than that in the 5 and 21-gram groups. The average increase in heat production for each group is shown in Figure 1, page 396.

#### SPECIFIC DYNAMIC ACTION

The increase in heat production due to 5.05 grams of levulose was on the average 0.5 calorie, which is equivalent to 3 per cent of the fuel value (heat of combustion) of the levulose; in the 10.1-gram series the increase of 1.8 calories equals 5 per cent. The summation of increases in the 15.5-gram group was 4.0 calories, which is equivalent to 7 per cent. In the 21-gram group, as pointed out in the discussion of the heat production, the increase was smaller in proportion to the amount ingested than in either the 10 or the 15.5-gram group, *i.e.*, only 1.5 calories, or 2 per cent. The summation of increases in the 26-gram group was 4.7 calories, which is 5 per cent of the energy value of the levulose ingested. In the discussion of the heat production, it was shown that to the positive increases above the basal value could be added about 4 calories which are due to the fact that the fall in the heat production from the fifth to the ninth half hour after 26 grams was not so large as that after 5 grams and intermediate quantities of levulose. Including this the amount of heat would raise the specific dynamic action for 26 grams of levulose to 9 per cent.

The increase in one of the experiments with 52 grams was 19.4 calories in  $3\frac{1}{2}$  hours, which equals 10 per cent, but the increase in the other experi-

ment was only 5.9 calories in  $3\frac{1}{2}$  hours, which equals 3 per cent. The average of the two would be 6.5 per cent of the fuel value. In neither experiment was the basal heat production reached after the ingestion of the levulose, so that the specific dynamic action is probably higher than the above values. The increase in the experiment with 104 grams was 45.8 calories in 4 hours, which is equivalent to 12 per cent, and this is minimum, as in the eighth half hour the heat production was still 4.4 calories above the basal value. Benedict and Carpenter (1918, p. 338) found, with 75 and 100 grams of levulose, 3 to 14 per cent, and Deuel (1927) has recently reported 7 per cent as the specific dynamic action of fructose. The specific dynamic action, then, rises for quantities of levulose varying from 5 to 15.5 grams, is low with 21 grams, and then rises somewhat more rapidly as larger amounts are given. There is more irregularity in the specific dynamic action of levulose than there is in the case of dextrose, but both sugars behave similarly in that the larger amounts result in greater increases of heat production on the percentage basis.

#### THE RÔLE OF THE WATER INGESTED WITH LEVULOSE.

The rôle which the water plays in the changes in carbohydrate metabolized and heat produced, when sugars are given, has already been discussed in the previous contribution on the effect of dextrose and water upon these factors. If correction were made for the effect of water alone in the results for 15.5 grams of levulose, or under, the increases in both the carbohydrate utilized and heat produced would be augmented. With 21 grams of levulose (200 cc. of water), the change in carbohydrate would be increased, and the change in heat produced would be decreased. With 26 grams of levulose (250 cc. of water), the increases in carbohydrate, though diminished, would not be affected seriously, and the increase in heat production would be diminished by 2.6 calories. Correction for the effect of water, when the levulose (52 and 104 grams) was given in 500 cc. of water, would lower the increase in carbohydrate by about 3 grams and would lower the increase in heat production by about 7 calories.

#### THE SIGNIFICANCE OF THE RESPIRATORY QUOTIENT AFTER INGESTION OF LEVULOSE.

One of the objects of this study was to determine whether the type of reaction observed after the ingestion of levulose is the same regardless of the amount given. A study of the respiratory quotients in these experiments shows that this is the case. There is a marked rise in the respiratory quotient to a peak, which takes place usually within 45 minutes. The main

difference in the results with the different quantities is the height to which the quotient rises and the duration of the period of the high quotients. If the basal respiratory quotient in each experiment is subtracted from the highest quotients for a single 10 to 15-minute period, the average rise in quotient for the different amounts of levulose is as follows: 5.05 grams, 0.05; 10.1 grams, 0.09; 15.5 grams, 0.18; 21 grams, 0.12; 26 grams, 0.16; 52 grams, 0.18; and 104 grams, 0.21. There is no marked difference in these increases when the amounts are above 10.1 grams, for 15.5 and 52 grams gave the same result. There is a tendency to longer periods of high respiratory quotients with increasing amounts ingested, the high quotients persisting for nearly two hours after 104 grams of levulose. Another fact of interest is that the rise in the quotient is independent of the basal respiratory quotient. In the 10.1-gram group, increases of 0.09 and 0.08 were found with basal respiratory quotients of 0.88 and 0.80; in the 15.5-gram group, a rise of 0.17 with basal quotients of 0.85 and 0.80; in the 21-gram group, increases of 0.13 and 0.12 with basal quotients of 0.83 and 0.87; and in the 26-gram group, a rise of 0.17 with basal quotients of 0.81 and 0.88, so that the reaction after levulose is uniformly independent of the original level of the respiratory quotient. High respiratory quotients after levulose, similar to those observed in the above experiments, have been found by nearly all previous investigators, most of whom have attributed the quotients to the process of formation of fat from levulose.

Joslin (1923, p. 233) was forced to the conclusion that the diabetic with respiratory quotient above unity converted levulose into fat. Dumont (1922) reported the beneficial effects of giving to diabetics those vegetables which produce levulose, and Root and Baker (1925) found increases in the respiratory quotient when levulose and Jerusalem artichokes were given to diabetic patients. Carpenter and Root (1928) demonstrated the absorption and utilization of the carbohydrates of the Jerusalem artichoke (presumably, for the most part, levulose and its polymers) in a diabetic patient with an absence of glycosuria, the prompt appearance of sugar in the urine when potato containing an equivalent amount of carbohydrate was given, and the equally prompt return to a sugar-free condition when the artichokes were again a part of the diet.

Campbell and Maltby (1928) have recently made a series of measurements of the carbon-dioxide combining power and lactic acid in the blood of patients to whom 100 grams of dihydroxyacetone, levulose, sucrose, glucose and maltose were given. They found that the ingestion of the first three substances resulted in a decrease in the carbon-dioxide combining power and an increase in the lactic acid of the blood, the greatest changes

occurring with dihydroxyacetone and the least with sucrose. On the other hand, they found little or no change after the ingestion of glucose or maltose. They conclude that the high respiratory quotients obtained with the first three materials were due to over ventilation and were not true metabolic respiratory quotients, whereas the quotients found after glucose and maltose were real indications of qualitative changes. However, it should be pointed out that compensatory decreases in the respiratory quotients are not found in the studies with levulose and sucrose. Other evidences in the literature of changes in the lactic acid in the blood when sugars were given conflict with the findings of Campbell and Maltby. Katayama (1929) found an increase in the lactic acid of the blood simultaneous with the hyperglycemia which took place after the ingestion of 1.75 grams of dextrose per kilogram of body weight in 22 normal and diseased subjects. Oppenheimer (1928) found no increase in the lactic acid of the blood either in normal persons or in patients with diseases of the liver when 50 grams of dextrose or levulose in 300 cc. of water were administered by mouth.

Douglas and Priestley (1924) have studied the changes taking place in the ventilation and the alveolar carbon dioxide in relation to the increase in the carbon dioxide elimination after the ingestion of 75 to 80 grams cane sugar. They found that the alveolar carbon dioxide remained practically unchanged throughout the periods of high respiratory quotients and that the percentage changes in the calculated alveolar ventilation were closely parallel to the percentage changes in the carbon dioxide elimination. They conclude that the respiratory center reacted to a uniform carbon dioxide level and that there was an adjustment of the ventilation to correspond to the changes in the carbon dioxide elimination. One is therefore justified in concluding that the high respiratory quotients which they obtained were true metabolic indices.

Part of the increase in oxygen absorption observed after levulose is ascribed by Campbell and Maltby to the increased work of over ventilation. Liljestrand (1918) found a maximum value of 0.7 cc. of oxygen per liter of increased ventilation, and the maximum increase in ventilation in any of the experiments in the series here reported was from an average of 5.7 liters per minute in the basal periods to 8.1 liters in the fourth 10-minute period of the experiment with 104 grams of levulose. If the above value for oxygen is applied ( $2.4 \times 0.7$ ), this increase in ventilation accounts for only 1.7 cc. of oxygen. The rise in oxygen consumption in this period was from a basal of 216 to 253 cc. per minute, an increase of 37 cc. Furthermore, neither the ventilation nor the oxygen fell materially until more than an hour and one half after the fourth period.

Campbell and Maltby comment that many of the high respiratory quotients after levulose are from isolated short periods and that one is not warranted in carrying out the calculations to a period of an hour from such short intervals of measurement. This comment applies neither to the investigations of Joslin nor to those here reported. Joslin's studies with diabetics were conducted with the patient in a respiration chamber (Benedict and Tompkins 1916) for successive half hour periods which usually totalled two hours, and our experiments were made in successive short periods with a total duration of more than two hours in the first series after the levulose was taken. In some of our experiments with the larger quantities, the high quotients were obtained for a period of an hour or more. In the experiment with 104 grams, the average non-protein respiratory quotient for 1 hour was 1.049 and for a half hour beginning 20 minutes later was 1.032. In view of this evident effect of levulose and of the fact that even small quantities gave large increases in quotients, it seems highly probable that the ingestion of levulose results in the formation of an oxygen-poor substance (fat) which may be either stored or subsequently burned.

The usual interpretation of respiratory quotients is that when they are over 1.00, formation of fat from carbohydrate takes place, but when they are below unity, the character of the metabolism may be either predominantly carbohydrate or predominantly fat, depending upon the level of the quotient. Benedict and Carpenter (1918, p. 248) have called attention to the possibility that formation of fat from carbohydrate may take place simultaneously with the utilization of the usual combination of protein, fat, and carbohydrate, even when the respiratory quotient is below unity. Cathcart and Markowitz (1927) have postulated that high respiratory quotients should be interpreted as indication of the formation of fat from carbohydrate and low quotients as an index of the opposite transformation. It is evident that if formation of fat from a particular carbohydrate takes place, it is an empirical interpretation of the respiratory quotient to conclude that this transformation occurs only when the respiratory quotient is above unity. Consequently, the probability of the formation of fat from carbohydrate, when the respiratory quotient is below unity, is not excluded.

#### SUMMARY

The respiratory exchange of a human subject was determined while he was in the post-absorptive condition and during  $3\frac{1}{2}$  to  $4\frac{1}{2}$  hours after the ingestion of levulose in amounts from 5 to 104 grams, which were taken in water at  $37^{\circ}\text{C}$ .



The maximum respiratory quotient was found mostly during the second half hour after levulose, and, with the larger quantities, the increases in the quotients were larger and continued later than with the smaller quantities.

The increase in apparent catabolism of carbohydrate represented 30 to 34 per cent of the amount of levulose given the subject, with the exception of 14 per cent in the experiments with 5 grams of levulose and 53 per cent in the experiments with 15.5 grams. Although the values, 29 and 31 per cent, were found for 52 and 104 grams, not all possible increases are included as the experiments were too short in duration.

Increases in heat production were found in all groups, which varied from 0.5 calorie in 1 hour after 5 grams of levulose to 46 calories in 4 hours after 104 grams. The minimum increase in proportion to the amount given was 1.5 calories in 2 hours after 21 grams.

The specific dynamic action varied from 2 per cent with 21 grams of levulose to 12 per cent with 104 grams, although in the latter case the total heat increment was not measured.

Although the values obtained for carbohydrate after levulose are treated mathematically as increases in metabolism of carbohydrate, a discussion is given with respect to the significance of the changes in the respiratory quotient, and the probability is favored that the reaction after levulose is one of conversion of carbohydrate into an oxygen-poor substance (fat), even when the respiratory quotient does not rise above unity.

#### BIBLIOGRAPHY

- Benedict, F. G., and Carpenter, T. M., 1918, *Carnegie Institution of Washington Pub. No. 261*.  
 Benedict, F. G., and Tompkins, E. H., 1916, *Boston Medical and Surgical Jour.*, CLXXIV, 857.  
 Campbell, W. R., and Maltby, E. J., 1928, *Jour. of Clinical Investigation*, VI, 303.  
 Carpenter, T. M., 1925, *Carnegie Institution of Washington Pub. No. 369*, 159.  
 Carpenter, T. M., and Root, H. F., 1928, *Archives of Int. Med.*, XLII, 64.  
 Cathcart, E. P., and Markowitz, J., 1927, *Jour. of Physiol.*, LXIII, 309.  
 Deuel, H. J., Jr., *Jour. Biol. Chem.*, 1927, LXXV, 388.  
 Douglas, C. G., and Priestley, J. G., 1924, *Jour. of Physiol.*, LIX, 30.  
 Dumont, J., 1922, *Bulletin de l'Academie de Medecine*, LXXXVII, 721.  
 Joslin, E. P., 1923, *Carnegie Institution of Washington Pub. No. 323*, 233.  
 Katayama, I., 1926, *Jour. Laboratory and Clinical Medicine*, XI, 1024.  
 Liljestrand, G., 1918, *Skand. Archiv. f. Physiol.*, XXXV, 244.  
 Lublin, A., 1926, *Archiv. f. exper. Pathol. u. Pharmacol.*, CXV, 101.  
 Oppenheimer, 1928, *Zeit. f. klin. Med.*, CVII, 467.  
 Root, H. F., and Baker, M. L., 1925, *Archives Int. Med.*, XXXVI, 126.

---

# EXPERIMENTAL NUTRITIONAL POLYNEURITIS IN THE RAT\*

BY M. R. SANDELS

(From the Department of Chemistry, Columbia University, New York)

Received for Publication—September 30, 1929

THE question has frequently been raised in recent years as to whether the rat is a suitable animal for use in studies of the antineuritic vitamin, since the symptoms of the specific deficiency (polyneuritis) have not in the past been produced with regularity in rats, during the course of feeding experiments, and because of this, doubt has arisen as to the value of this technique. As recently as 1928 Kinnorsley, Peters and Reader<sup>1</sup> make the statement that "it is not yet absolutely certain that rat tests can differentiate the curative factor in the vitamin B complex." Chick and Roscoe<sup>2</sup> rarely observed symptoms of polyneuritis in their animals on their basal diet K alone. Drummond<sup>3</sup> noted paralytic symptoms in three only out of a large number of rats on diets deprived of water-soluble vitamins. Opposed to these negative results, we have the earlier work of Schaumann<sup>4</sup> who found that rats confined to a diet of denatured horse flesh developed symptoms of paralysis. He states that three rats on this diet became lame after 25-28 days on this diet. All three animals survived in this condition for the eighty days of the experimental period. Hofmeister<sup>5</sup> was able to produce symptoms of polyneuritis in rats with considerable regularity. His work will be referred to again.

With the recent differentiation of the antineuritic vitamin from the more heat-stable substance (factor P-P, Vitamin B<sub>2</sub> or G) it becomes important to study the response of experimental animals to varying degrees of restriction of the vitamin, and to discover whether or not a characteristic train of symptoms may be produced in the rat, which may

\* Published as Contribution No. 620 from the Department of Chemistry, Columbia University.

<sup>1</sup> Kinnorsley, H. W., Peters, R. A., and Reader, V., Antineuritic Yeast Concentrates. III. The Curative Pigeon Test: A Critique. *Biochem. Jour.*, 1928, **xxi**, 276.

<sup>2</sup> Chick, H., and Roscoe, M. H., On the Composite Nature of the Water-Soluble B Vitamin. *Biochem. Jour.*, 1927, **xxi**, 698.

<sup>3</sup> Drummond, J. C., A Study of the Water Soluble Growth-Promoting Substance in Yeast L. *Biochem. Jour.*, 1917, **xi**, 255.

<sup>4</sup> Schaumann, H., Further Contribution to the Etiology of Beriberi. *Trans. Soc. Trop. Med. Hyg.*, 1911, **v**, 58. Cited by Hofmeister.<sup>5</sup>

<sup>5</sup> Hofmeister, F., The Beriberi of Rats. *Biochem. Zeitschr.*, 1922, **cxxviii**, 540.

be correlated with the severity of the deprivation of the vitamin in the diet.

The observations reported here were made in the course of experiments designed to measure the solubility of the antineuritic vitamin in alcohol 80 per cent by weight.<sup>6</sup> Over 100 animals were kept under controlled conditions for periods varying from a few weeks to several months. Their response, when graded additions of the antineuritic substance were made to the basal diet, has been carefully noted.

#### EXPERIMENTAL METHODS

Healthy young rats of known nutritional history were placed at 28 to 29 days upon a diet believed to be quite free of the antineuritic vitamin but adequate, so far as present knowledge goes, in all other respects. The diet of Sherman and Spohn<sup>7</sup> for determination of vitamin B (using this term in the older sense of the word) was modified to include 15 per cent of dry bakers' yeast, autoclaved for three hours at 125°C. The autoclaved yeast replaced an equal weight of cornstarch and supplied the recently differentiated heat-stable factor, but, so far as feeding tests indicated, contained no important amount of the antineuritic vitamin.

Animals received this basal diet with or without the quantitatively graded additions of materials whose antineuritic potency was to be tested. These materials were dry bakers' yeast, alcoholic extracts of yeast, the residue left after this alcoholic extraction, and an alcoholic extract of ground whole wheat. In the preparation of the alcoholic extract and the extracted residue of yeast,<sup>8</sup> 400 grams of air-dry bakers' yeast were treated with 1500 cc. of alcohol (80% by weight), thoroughly stirred and allowed to stand at room temperature (20° to 25°C.) for 24 hours; then filtered with suction, and the yeast washed on a Buchner filter with 750 cc. of alcohol of the same strength; then again stirred with 1500 cc. of the alcohol, allowed to stand 24 hours, filtered and washed as before. The residue was dried in the air at room temperature. The extract obtained by combining the two filtrates and the washings was a clear yellow solution of  $p_H$  6.1. It was concentrated on the steam bath and evaporated at

<sup>6</sup> Sandels, M. R., Dissertation, Columbia University, 1928.

Sherman, H. C., and Sandels, M. R., Further Experimental Differentiation of Vitamins B & G (unpublished).

<sup>7</sup> Sherman, H. C., and Spohn, A., A Critical Investigation and an Application of the Rat Growth Method for the study of Vitamin B. *Jour. Amer. Chem. Soc.*, 1923, *xlv*, 2719.

<sup>8</sup> Sherman, H. C., and Sandels, M. R., Experiments with reference to the more heat-stable factor in the vitamin B group (Factor P-F, Vitamin B<sub>2</sub> or G). *Pro. Soc. Exper. Biol. Med.*, 1929, *xxvi*, 536

room temperature on to cornstarch. The dried product was ground and enough cornstarch was added to make the final weight of the preparation 200 grams.

#### SYMPTOMS OF POLYNEURITIS IN THE RAT

Animals on the basal diet alone grew somewhat during the first 7 to 14 days then declined rapidly and died within 25 to 40 days' time, the average survival period being 38.1 days. The majority of these drooped in their cages and became weak and unsteady on their feet, but rarely showed characteristic symptoms of polyneuritis. At death cyanosis was usually prominent.

Animals receiving the vitamin in amounts which were measurable, but which were insufficient for protection, developed almost without exception, typical symptoms of polyneuritis. Head retraction was usually one of the earliest symptoms. In its mildest form it appeared as a nervous jerking of the head when the animal became excited. In more aggravated cases, the opisthotonic position of the head was quite marked. There was sometimes complete paralysis of the hind legs, but more frequently the nervous involvement manifested itself in a spastic gait, or in loss of muscular control, especially when handled. Animals were apt to hold their heads on one side and walk in circles. The survival period appeared to be correlated with the amount of the vitamin supplied.

An amount of the vitamin which allowed approximately net maintenance of the weight of the experimental animal over an eight week period, was frequently insufficient for protection from the deficiency. At maintenance level a subacute or chronic type of polyneuritis often developed. Animals in this condition usually maintained life for several weeks, and the majority of these showed definite symptoms of polyneuritis continuously after the deficiency had once become manifest.

A few typical protocols of animals showing characteristic behavior are given below:

1. Protocols of litter mates, showing graduation of symptoms when fed graded amounts of antineuritic vitamin. The basal diet used in these experiments, a modification of the Diet 107 of Sherman and Spohn had the following composition:

Casein, extracted with 60% alcohol.....	18%
Salt mixture (Osborne and Mendel).....	4%
Cod liver oil.....	3%
Butter fat, filtered.....	7%
Cornstarch.....	53%
Autoclaved bakers' yeast (3 hrs. at 125°C).....	15%

*Rat B2406.* Put at 28 days of age upon the basal ration designed to be devoid of antineuritic vitamin, without further addition. During the first week it gained somewhat, then declined rapidly and died without noticeable symptoms of polyneuritis, on the seventeenth day of the experimental period.

*Rat B2403.* Put at the same age upon the same basal ration but received six times weekly, as supplement, the alcoholic (85%) extract from 0.400 gram whole wheat. During the third week the animal developed acute symptoms of polyneuritis, the hind legs being completely paralyzed. It died within a few days.

*Rat B2402.* Put at 28 days upon the same basal ration with supplement six times weekly of the alcoholic (85%) extract from 0.800 gram whole wheat. The animal grew considerably during the first two weeks, then lost slightly. During the fourth week polyneuritis developed and coincidentally there was a sharp loss in weight. Following this there was a slow gain in weight, although pronounced symptoms of polyneuritis were present continuously and included head retraction, spastic gait and marked excitation. This latter manifested itself in the animal's frantic circling of the cage whenever there was movement or noise in the laboratory. Four weeks later (the end of the eighth week of the experiment) the animal died in convulsions.

## II. Protocols of animals showing typical subacute or chronic polyneuritis.

*Rat L129.* 12/3/27 Put at 28 days of age on the same basal diet, designed as devoid of antineuritic vitamin, plus 0.200 gram yeast six times weekly. 1/4/28 Head retracted. 1/9/28 Spastic gait, head retracted. 1/13/28 Cartwheel turning, drags hind leg. 2/3/28 For past five weeks animal has showed continuously the classic symptoms of polyneuritis, spastic gait, head retraction and convulsive seizures when handled. Chloroformed.

*Rat 23065.* 12/12/27 Put on the same basal ration plus the extract from 0.400 gram yeast three times weekly. 1/19/28 Polyneuritic. 1/30/28 Very weak and sick, partly paralyzed. 2/6/28 Condition improved, more lively, better control of movements. 2/13/28 Paralytic seizures when handled. Chloroformed

## DISCUSSION AND CONCLUSION

This subacute or chronic type of polyneuritis has seemed of particular interest. Vedder and Clark<sup>9</sup> in 1912 described a similar condition in fowls and their findings were later confirmed by Weill and Mouriquand.<sup>10</sup> Recently Randoin and Lecoq<sup>11</sup> reported a similar condition in a pigeon receiving an amount of vitamin insufficient for full protection. Hofmeister<sup>6</sup> described this subacute type of polyneuritis, and, as in the present study, found the condition associated with shortage rather than complete absence of the antineuritic vitamin. The present work confirms that of Hofmeister and extends it somewhat in showing a definite gradation of

<sup>9</sup> Vedder, E. B., and Clark, E., A Study of Polyneuritis Gallinarum: A Fifth Contribution to Etiology of Beriberi. *Philippine Jour. Sci.*, Sect. B. 1912, vii, 423. Cited by Funk, C., *The Vitamines*, 1922.

<sup>10</sup> Weill, E., and Mouriquand, G., Experimental Chronic Beriberi Syndrome. *Compt. rend. soc. biol.*, 1918, LXXXI, 423.

<sup>11</sup> Randoin, L., and Lecoq, M. R., Polyneurite et Scorbut Chroniques. *Bull. soc. chim. biol.*, 1927, ix, 513.

symptoms correlated with the extent of the deficiency of the vitamin in the food supplied.

It seems probable that the irregular appearance of symptoms in earlier experiments was due to differences in the basal diets used and to the failure to recognize the need for inclusion of the heat-stable vitamin G. Results secured in the present study leave no reasonable room for doubt that the rat is sensitive to the specific deficiency and will develop well marked symptoms of polyneuritis, with sufficient regularity to be a valuable aid in determining the extent of the deficiency in antineuritic vitamin to which the animal is subjected.



---

## FURTHER EXPERIMENTS ON THE INFLUENCE OF FOOD UPON LONGEVITY\*

By H. C. SHERMAN AND H. L. CAMPBELL

*(From the Department of Chemistry, Columbia University, New York)*

Received for Publication—October 2, 1929

THE available data regarding noteworthy cases of longevity in man have given the impression that this depends almost entirely upon inheritance. The limitations of the method of investigation in such cases, however, may quite conceivably have tended to obscure or minimize the importance of food, and perhaps of other factors. In compiling the available facts regarding a person who has attained to great age, the ages reached by parents and grandparents will usually stand out as clear-cut quantitative data; while it will be quite impossible to obtain equally clear-cut and quantitative data as to the food consumed throughout a life-time.

This latter factor can, however, be studied experimentally by the use of such a laboratory animal as the rat; and the suitability of this species for use in studies of nutritional problems of human importance has been fully discussed by Osborne and Mendel (1), while further evidence is afforded by the work of Folin and Morris (2) which showed close similarity of the end products of metabolism in the two species.

McCollum and others (3) have repeatedly shown that the normal length of life of experimental animals (rats) may be shortened in almost any desired degree by dietary deficiencies of varying degrees of severity.

Our own problem has been to determine whether, starting with a food supply and nutritional condition already adequate and normal, it is possible by improvement of the food to induce a definite increase in longevity.

In the present experiments the original diet (Diet A) consisted of a mixture of one-sixth dried whole milk and five-sixths ground whole wheat, with table salt and distilled water. Families of experimental rats are still thriving after 21 successive generations on this diet. This is probably an unprecedentedly rigorous test of the adequacy of a food supply and shows beyond question that this original diet was certainly adequate.

Simultaneously, parallel lots of animals, of the same heredity, have been fed a diet differing only in that the proportion of milk powder in the food mixture was increased to one-third (Diet B).

While Diet A was clearly adequate, Diet B proved to be better, and

---

\* Published as Contribution No. 618 from the Department of Chemistry, Columbia University.



induced, in addition to other evidences of improved nutrition (4), *both* a decrease in infant mortality *and* an increase of ten per cent in the average length of life of the adults.

The two groups of animals, which as already mentioned were of the same heredity, were kept under identical conditions in all respects except for the difference in food. The higher proportion of milk in Diet B means chiefly, in chemical terms, a richer intake of calcium, of vitamins A and G, and of certain of the amino acids. Experiments designed to determine to which of these chemical factors the increase of longevity is attributable are now in progress but not yet sufficiently advanced to permit of final conclusions. From the results thus far obtained it seems probable that the increased intakes of calcium, of vitamin A, and of vitamin G may all have contributed to the higher degree of health and the increased length of life.

That the increase of longevity was real and not accidental is made clear both by simple statistical treatment of the data and also by grouping them in such manner as to afford a series of comparisons (of the animals on the two diets) in terms of the percentages attaining to different degrees of longevity. The evidence as assembled in these two ways is briefly summarized in Tables I and II, respectively.

A part of this evidence was presented in a preliminary report of about a year ago (5). Since that time the numbers of animals compared have been considerably increased and the findings have become more conclusive.

TABLE I  
COMPARISON OF LONGEVITY OF RATS ON DIETS A AND B

	Diet A		Diet B		Difference of Length of Life in Days
	Number of cases	Average Length of life in days	Number of cases	Average Length of life in days	
Males	135	571 $\pm$ 8.0	124	635 $\pm$ 8.5	64 $\pm$ 11.7
Females	196	603 $\pm$ 8.0	163	669 $\pm$ 7.8	66 $\pm$ 11.2

The average lengths of life and the differences of these averages, as given in Table I, are followed by estimates of their probable errors computed in the usual manner. While it is true that such computation is based upon an assumption of symmetrical frequency distribution and therefore may or may not be precisely accurate for data of this particular kind, yet, inasmuch as these data belong to the category of natural phenomena to which, according to Reitz and Mitchell (6) the usual calculations of probability may normally be expected to be applicable, there is good reason

to believe that the quantitative relations thus computed are at least approximately correct. This being the case, and the increase of longevity being 5.6 and 5.9 times its probable error, for males and females respectively, it follows that, speaking mathematically and from the standpoint of this mode of interpretation alone, the chances are about ten thousand to one (10,000:1) that the increase in longevity here found is a true difference due to the food, and not an accidental difference nor due to unknown causes.

The findings are established with still greater certainty when our basis of scientific judgment is broadened to include a further consideration of the data from the point of view summarized in Table II. This makes possible a series of nine comparisons, and in every one of these the greater longevity attained upon Diet B than upon Diet A is clearly apparent.

TABLE II  
INFLUENCE OF FOOD UPON ATTAINMENT OF DEFINITE AGES

Percentage of Lives Longer than:	Males		Females	
	On Diet A	On Diet B	On Diet A	On Diet B
600 days	42.9	65.3	54.1	73.0
700 days	14.8	32.3	27.6	43.6
800 days	2.9	10.5	12.2	15.9
900 days	0.0	1.6	2.6	5.5
1000 days	—	—	0.5	1.2

Hence it may be regarded as established beyond any reasonable doubt that, starting with a diet which is already clearly adequate, it may still be possible to induce a very significant improvement in longevity by enriching the diet in certain of its chemical factors.

#### REFERENCES

1. Osborne, T. B., and Mendel, L. B., Publication No. 156, Carnegie Institution of Washington (1911) and subsequent papers in the *Journal of Biological Chemistry*.
2. Folin, O., and Morris, J. L., *Jour. Biol. Chem.*, 1913, XIV, 509-515.
3. McCollum, E. V., Simmonds, N., and Parsons, H. T., *Jour. Biol. Chem.*, 1921, XLVII, 111-246. McCollum, E. V., and Simmonds, N., "The Newer Knowledge of Nutrition," 3rd Ed. 1926.
4. Sherman, H. C., and Campbell, H. L., *Jour. Biol. Chem.*, 1924, LX, 5-15.
5. Sherman, H. C., and Campbell, H. L., *Proc. National Academy of Sciences*, 1928, XIV, 852-855.
6. Reitz, H. L., and Mitchell, H. H., *Jour. Biol. Chem.*, 1910-11, VIII, 297-326.





## Editorial Review\*

### BIOLOGICAL VALUES AND THE BEHAVIOR OF FOOD AND TISSUE PROTEINS<sup>1</sup>

ONCE read, somewhere, of two wanderers, one a German, who came to a fork in their road. To the left the arrow read, "To Paradise"; to the right, "Problems on Paradise." The German, naturally, chose the latter.

Consistently following this national characteristic of the Germans, I must not be expected to give a detailed summary of the biological values of all possible pure proteins, or of the synthetic and naturally occurring protein mixtures; or of the practical results which have been achieved through the knowledge of these facts in the nourishment of sick and healthy persons, in the breeding of domestic animals, and in the production of milk, meat, eggs, etc.

#### I. BIOLOGICAL VALUE OF FOOD PROTEIN

I would rather discuss the methods employed in the determination of biological values and stress particularly the individual factors which influence the practical value of a protein. In this connection, I wish to criticize the methods. The one method which uses the nitrogen equilibrium(balance) experiment, I should like to call the analytical method. By means of this method determinations on human beings can be carried out. The other makes use of an artificial diet consisting of pure nutritive elements, with which small experimental animals are kept alive or in a growing condition. I should like to call this latter method the synthetic one.

The synthetic method gives us a definite answer to the question whether the protein is useful or not and permits an approximate measure of the

\* The writer, Professor Karl Thomas, was for many years associated with Professor Max Rubner in the Hygienic Laboratory and later in the Physiological Institute at the University of Berlin. He was for three years Assistant in Chemistry at Tübingen and made his habilitation for physiology at Greifswald with Bleibtreu in 1912. He returned in 1915 to Berlin where he was in charge of the Kaiser Wilhelm Laboratory for Arbeits-Physiologie under the general direction of Prof. Rubner. Since 1921 Dr. Thomas has been Professor of Physiological Chemistry at the University of Leipzig, sharing the chair originally occupied by C. Ludwig. While in Rubner's laboratory Dr. Thomas introduced a method for establishing the biological value of different proteins. With himself as subject, he studied the effects on nitrogen excretion of ingesting different protein foods, in comparison with a diet containing no protein. From these results he was able to give numerical values to different kinds of protein. This conception of biological value depending on composition has become thoroughly established in the physiological literature. Prof. Thomas also has contributed important researches in intermediary metabolism, such as the significance of creatin and the beta-oxidation of  $\alpha$ -amino fatty acids. He has sought in many studies to make the methods of synthetic organic chemistry serviceable to the physiology of metabolism. With Knoop of Tübingen he is now editor of the well-known Hoppe-Seyler's Zeitschrift für Physiologische Chemie.—Ed.

<sup>1</sup> Revised from an address delivered at the University of Rochester Medical School, September 30, 1929.

usefulness through a comparison of the amounts of various proteins necessary for maintenance or growth.

With the nitrogen balance method, on the other hand, the research worker obtains more nearly quantitative results, since its basis is the definite bodily requirement which Rubner has called the wear and tear factor.

What is it that is designated as the practical or biological value? I can explain it perhaps most simply by means of the figure on page 421. I plot the grams of nitrogen which express the bodily requirement on the ordinate O-C; on the abscissa I plot the diet nitrogen. Through C a line is drawn parallel to the abscissa on which I plot the nitrogen excreted, E,  $E_1$ ,  $E_2$ . When we have nitrogen equilibrium the connecting line between the end points of intake and excretion E-I is at right angles to the abscissa and parallel to the ordinate. When the balance is positive, then this connecting line  $E_1$ -I leans to the left, toward the ordinate; when it is negative,  $E_2$ -I leans to the right, away from the ordinate.

The least nitrogen with which I can achieve a balance just manages to cover the nitrogen requirement;  $100 \times \tan \alpha$  expresses the relation in which the diet nitrogen can replace the requirement; in other words, the biological value. If the food protein is exactly equal to the body protein destroyed in katabolism then we have  $O-I = C-E = O-C$

$$\alpha = 45^\circ \text{ and}$$

$$100 \times \tan \alpha = 100$$

Let us study this picture still further. The distance O-C represents the need of N on a diet which consists mainly of carbohydrates and contains no protein. The entire requirement must then be cared for by the body's own nitrogen; the protein catabolism under these circumstances being the lowest possible. Although I am not convinced that the N excretion actually represents the protein metabolism under the circumstances of the nitrogen minimum, nevertheless we judge the protein requirements by the N excretion in the usual manner.

Is the distance O-C really constant? It consists of three components: The urine N (u) the feces N (f) and the N which leaves the body by means of the skin. The last of these cannot always be neglected. When, for instance, in the case of heavy physical labor, the sweat glands are more active, it is possible for the sweat to contain 0.2 gm. more N per hour than when the body is at rest (F. G. Benedict, 1906). One must bear in mind, however, that this amount rises and falls only in direct proportion to the rise and fall of the N metabolism in the entire body. At the level of the

N minimum it may be neglected, since it amounts only to 0.13 gm. per day (Thomas, 1910).

The feces N ( $f$ ) is present not only on the ordinate as part of the N requirement, but on the line parallel to the abscissa as part of the N excretion. It consists of two parts; 1st, the exogenous portion, derived from the protein intake, and 2nd, the endogenous portion derived from the secretions of the gastro-intestinal glands. Both are variables, and are dependent upon, 1st, the kind of food protein, and 2nd, on the character and quantity of the ingested food as a whole. In the N minimum experiments, which I conducted, the ingested N was kept at an approximately constant

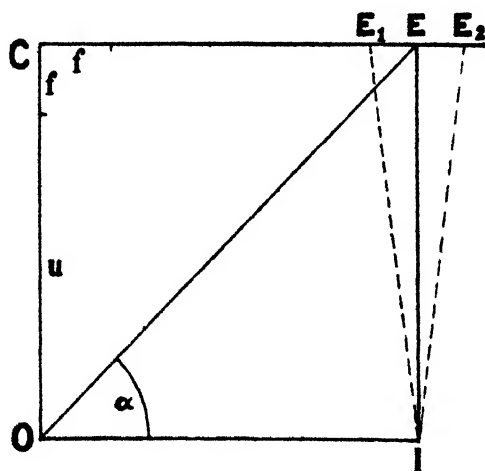


FIGURE 1.

quantity. In addition to this precaution, the foodstuffs that were absorbed with difficulty could be avoided.

The excretion C-E must, naturally, be entirely free of N-containing residues from previous protein-rich diets. These must be removed by a sufficiently long preliminary period. To this end a week at most sufficed, as I proved on myself, and as was later shown by Martin and Robison (1922) in detailed observations. Only in the case of very great over-feeding of protein does the return of excretion to the wear and tear quota require a longer time (as much as 2-3 weeks) (Thomas, 1910). This is probably due to the fact that under the circumstances not only is a removal of N residues involved, but also a complete destruction of protein reserves. That which finally remains in the tissue as extractive N is not residue but, according to Mitchell, Nevens and Kendall (1922) a definite component of the organism.

Thus far we have been considering only the katabolism of body protein in the case of protein starvation, in other words, the nitrogen requirement. We, have, after a fashion, concerned ourselves only with the ordinate. Let us now turn to the abscissa. In any experiment to determine the biological value, the body requirement should be just balanced by the food protein. I must, therefore, achieve a minimum under the following conditions:

1. Nitrogen equilibrium, or balance  $\pm 0$ ; that is, when I-E is at right angles to the abscissa. This is important, for in the case of a negative balance when the body contributes protein of its own, it is possible that that protein will be the one most necessary and least present unit—in other words, the line E-E<sub>2</sub> represents nitrogen of a new quality! The body can, perhaps, to a certain extent, do without one or two of the important proteins. On that subject we cannot say anything definite because we do not know enough about the composition of the protein reserve. If the above mentioned possibility should arise, then we have the case of a less important protein taking an important part. But this is possible only for a limited, short time. It is out of the question for any longer period simply because it would then be necessary for the remaining body protein to take on a different constitution (Neubauer, 1929).

2. The lowest possible N balance. How shall I accomplish this? I move from the left along the abscissa, that is, I use too little food protein so that the balance is negative. I then add protein daily in small amounts until the connecting line E-I is at right angles to the abscissa. This method is better than the one involving a start with a larger amount and daily decreasing the intake. The danger of protein residues remaining is too great. How is it in the case of such incomplete proteins as gelatine? I intentionally did not study them. In that case O-I would be  $\infty$  and tangent  $\alpha$  then = 0. Robison (1922) later confirmed this theory on himself.

3. The length of the distance O-E depends also upon the speed of absorption. The proteins are not suddenly and completely split into free amino acids in the gastro-intestinal tract. This takes place in steps as has been shown by artificial ferment hydrolyses. Therefore, every moment sees a different mixture of amino acids absorbed, or, in other words, a protein of a different biological value. This, however, is minimized by the action of the pylorus which permits the passage of food only in small portions. Thus, as the most difficultly split acids are set free, and absorbed only in the lowest portion of the ileum, we have at the same time an absorption of the more easily set free acids, from a portion passing later through the pylorus, in the duodenum and upper jejunum. All of the

amino acids, which are absorbed at the same time, meet again in the portal vein. The proportions of the amino acids to each other are not changed by the slow absorption of some of them, except perhaps slightly at the beginning and at the end of the process. The amino acids in the diet should take care of the body's requirements for a period of 24 hours! The question is whether this requirement shows a regular daily variation. Perhaps only a deviation for definite amino acids? The possibility is logical; we know nothing of the facts and are at present in possession of no instrumentality which will make the point clearer.

Do the absorbed amino acids cover the need of the last few hours of the day? Ordinarily yes, simply because we invariably consume more protein than we require. The question is whether this is also the case in the small amounts involved in our minimum. Of that we are not certain. I have therefore distributed the feeding of the protein over the entire day. In the case of many, especially plant foodstuffs which require a longer time for mastication and digestion, this is taken care of automatically; but with such rapidly absorbed foods as meat, milk, etc., one must attain this advantage through fractional feeding.

The ability of the liver to store protein must also be considered in this connection (Grund, 1910; Berg, 1922, 1926).

We have now discussed all the theoretical difficulties which lie in the way of the determination of the food value of a protein. I can, perhaps, summarize them best in the words of Miss Jordan Lloyd, a student of Sir Gowland Hopkins. She says "The idea of expressing the value of different food proteins on an arithmetical scale is theoretically sound, but the practical method by means of which these values are to be obtained have been the subject of much discussion." I will only briefly present the following extracts from my results.

The proteins in potatoes fulfill our needs better than those in bread; finest wheat flour contains a less important protein. In contrast, milk presents a very important product, not only to fulfill the requirements of addition, but also those of turn-over. That need not astonish us, when we recollect that in the case of the rapidly growing calf the turn-over or the wear and tear quota comprises an appreciable portion, more than half, of the entire required protein. Milk is also probably better adapted to the needs of the grown-up than meat. Muscle meat represents only a single organ adapted to a definite function and requiring specific protein material; the wear and tear quota represents, however, the common wastes of all the organs of the body. Later workers have confirmed these results.

I believe I have now covered everything on the N balance method for



the determination of the biological value of the proteins, at least everything that can be called a definite result. We have in addition criticized and, I trust, objectively, the method in all details. I should now, however like to call attention to two additional points.

First, to the fact that comparatively few studies have been made where this method has been used. Very often, and especially here in this country, proteins have been examined only in rat-feeding experiments or, in other words, by the synthetic method, as I called it, in contrast to the analytical or balance method. Why is this the case? Martin and Robison write that the N balance type of experiment seems simple, but is not so in fact! They are right indeed! Accurate N minimum experiments can rarely be carried on for a sufficiently long period. It is possible with bread, potatoes, rice, corn and similar food stuffs, which are naturally of high starch content, but not with a diet which consists mainly of the unappetizing pure starch, milk sugar and cane sugar. I am more firmly convinced to-day, than twenty years ago, that the N-free control diet is necessary but that we can supplant the sugar to a great degree by pure fat and thus achieve a more tasty and varied diet (Zeller, 1914; Murlin, 1907).

The synthetic method presents the advantage that experiments can easily be carried out for any length of time and that the proteins can be tested on growing as well as grown animals and in addition on a large number of animals. The results give therefore good average values. Still another advantage is the fact that the long-continued experiments, which often require a great deal of expensive pure proteins can be carried out by the use of small experimental animals. The disadvantage lies in the fact that in lengthy experiments vitamin preparations must be administered which are not entirely free from protein or amino acids.

Secondly, I should like to point out that I have not myself carried these experiments further—I do not know whether anyone has noticed it; I never found any indication to that effect in the literature! This, however, seems to me to be the proper time to explain myself. The nitrogen balance method has a purpose when one desires an arithmetical expression for the biological value of a protein; the synthetic method yields merely approximate comparative values. But the arithmetical value can very easily vary by the addition of small amounts of any of the rarer proteins; or, differently expressed, the value changes noticeably when a less important protein is completed by the addition of some other one. In the meaning of "completion" we can already see the possibility of two very unimportant and valueless proteins combining to form a mixture of extremely

high value. That was clearly shown through the beautiful experiments of Osborne and Mendel (1915). Gliadin alone does not produce growth in young rats but can keep them at constant weight. Gelatine cannot even produce these results. The two together, however, guarantee excellent growth. All that was necessary was the enriching of the almost lysin-free gliadin with the relatively lysin-rich gelatine. The enriching of white wheat flour with powdered milk, as performed by Sherman (1924) is another example. There are many similar combinations. It should be mentioned that the natural foodstuffs never contain only one single protein. In milk, casein is enriched by lactalbumin; in bread prolamine by glutenine. You can see, therefore, that while the physiologist might be interested in determining the biological value of any single pure protein, the hygienist, who must deal with the practical side of the question, must bear in mind that the value cannot be obtained from the sum of the values of the individual components of the diet, but rather, the value must be experimentally determined for each different diet. The human, moreover, changes the nature of his diet from day to day. Average values for him are out of the question. It is quite another thing in the feeding problems for domestic animals. In this case such experiments have a real practical value and have been thoroughly studied here in the United States from the viewpoint of national consumption and national development.

I should like to compare the numbers indicating the biological values of proteins with the numbers well known as the fat constants. Both numbers are of practical value; they help in scientific research work, but they are not a part of it.

What we need to know is:

1. Which amino acids must be present in the food,
2. How much we require of each,
3. And to what purpose.

These questions deal with the carbon skeleton of the amino acids, and not with their N content. The N is the least important of the elements composing protein. Because of the ubiquity of the Kjeldahl method and the lack of methods to determine the different carbon skeletons, we deal with the N; but we should not do so. Armed in the future with knowledge of the behavior of the carbon skeletons we shall be able to calculate the value of every protein mixture as it occurs in a diet. The duty of the scientist is to furnish the fundamentals to the practitioner, and to make certain of these fundamentals is in particular the duty of the physiologist.

## II. BEHAVIOR OF FOOD AND TISSUE PROTEIN IN METABOLISM

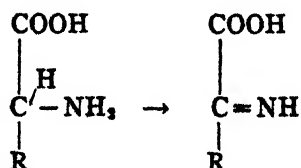
The idea of "food protein" must apparently surrender to the name "amino acid mixture". Does the same apply to the idea of "tissue protein", that is to say, are the katabolic processes the same in both cases? I am using the term tissue protein to cover the protein of the living substance of all tissues. This excludes all of the "dead", enclosed, reserve material of the cells as, for instance, all of the extractives and excretions and intermediary products between the cell walls.

As food protein we must include all those amino acids which do not serve for replacement in the wear and tear factor or, in other words, that portion which we labeled as superfluous.

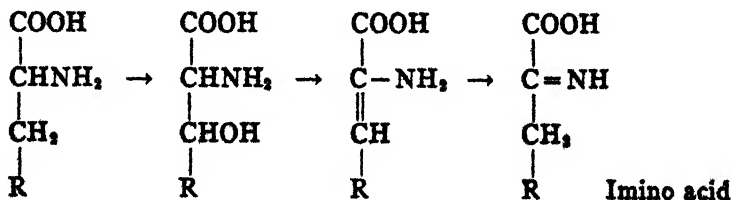
These amino acids are deaminized. The N-free carbon chains are either further synthesized or stored as sugar or, under special circumstances, are excreted as acetone or completely oxidized.

The deamination belongs to the first processes in katabolism and takes place in the liver (Mann and Magath, 1924).

Let us follow the process of deamination in single steps. It proceeds either by means of oxidation or reduction, as has been shown by Knoop, Dakin, Neubauer, Embden and Wieland. We can write the formulae as follows:



According to Dakin (1926), however, we can also write the reaction thus:



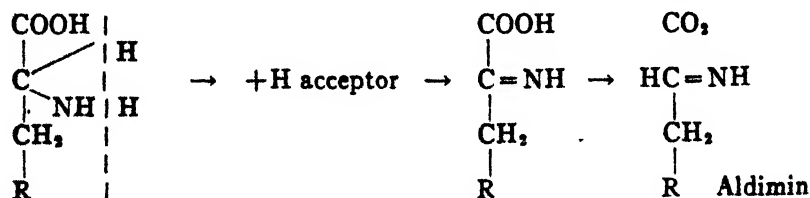
At this point one must recollect, however, that  $\alpha$ -amino,  $\beta$ -hydroxy acids have never been isolated as free acids but always as a component of protein. Be that as it may, the important fact is that the imino acid is an intermediate product in both cases.

What is the next step? Does the deamination by hydrolysis come first and is then followed by the loss of carbonic acid, or do the steps take

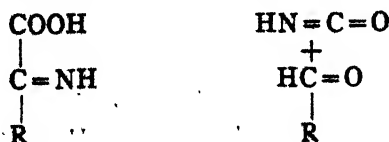
place in the reverse order? Or perhaps, as a third possibility, simultaneously?

Until a few years ago, it was believed that only the alpha keto acids were the first intermediate products, for these acids probably often resulted in the katabolism brought about by yeast. In normal as well as abnormal conditions of metabolism of animals and humans we have a complete parallelism between them and the respective amino acids, and this is true moreover of foreign as well as of native amino acids. By the feeding and blood perfusion of foreign amino acids it has been possible to isolate, and very often in large amounts, the respective keto acids. In spite of this it is my opinion that these are only probable proofs and that one must be extremely careful in the biological sciences in drawing analogies from such results. For perhaps the keto acid can only be isolated, or possibly occurs only, when the work is carried out under unnatural conditions with a carbon skeleton foreign to the body and with a surviving, that is to say, a dying organ, or by flooding of the animal body or the yeast cell with the amino acid under conditions which would never occur naturally.

I should, therefore, rather draw the conclusion that only under these unnatural conditions does deamination precede decarboxylation. We can draw no conclusions as to the normal procedure from these experiments. Wieland and Bergel (1924) oxidized the amino acid catalytically with pulverized palladium and animal charcoal. Under these circumstances decarboxylation preceded deamination, so that aldimin occurred as an intermediary product. They bring up the question whether the katabolism in the animal body does not also take the same course, but they feel it is not to be answered by analogous experiments with biological materials.



It is also possible for deamination and decarboxylation to occur simultaneously after the manner of the splitting off of cyanic acid.



These deductions help renew the old hypothesis of F. Hofmeister concerning the oxidative urea formation. Fearon (1926) supplied the new equations using the work of E. A. Werner on the constitution of urea as a basis.

According to this theory the carbamid form  $O=C\begin{smallmatrix} \nearrow NH_2 \\ \searrow NH_2 \end{smallmatrix}$  does not exist,

but instead a cyclic acid  $HN=C\begin{smallmatrix} \nearrow NH_2 \\ \searrow O \end{smallmatrix}$  in which the cyanic acid is already

formed. Thus we apparently must return to the 100-year old Wöhle<sup>r</sup> urea synthesis; Hoppe-Seyler and Salkowski permitted it to stand as regards the animal body. Frankly, cyanic acid has never been definitely proved to be present in the blood, opposing Montgomery's view (1925), and what is more, it cannot be so long as the methods in use remain as incomplete as they are to-day.

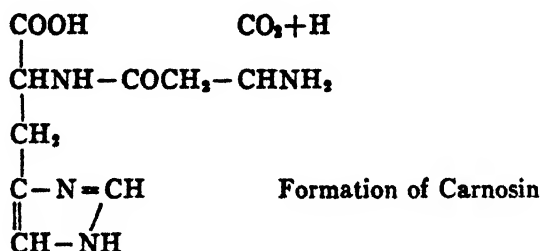
To sum up; the main process involved in the catabolism of the *superfluous*, ingested amino acids is the building of fatty acids with one less carbon. Oxidation, deamination and decarboxylation are three steps of a chain reaction. In my opinion, the shortening of the carbon skeleton right at the beginning is of great biological importance. Only this process renders it impossible for the carbon residue to be used in the synthetic preparation of amino acids. The amino acid is thus completely removed from the intermediary protein metabolism.

The nitrogen, to further develop these ideas, plays a special part because it is the presence of the amino group which diverts the entire amino acid to that place where it may be used. That is to say, to the point at which a deficiency has arisen through the wear and tear of the tissue protein and, moreover, where the deficiency can be corrected only by that one amino acid. The amino group, therefore, protects the entire acid from being split up outside of the liver, at least in mammals. In the dog which has had its liver removed, the amino acids, according to Mann and Magath, pile up in the blood. In birds ammonia can be split off even when the liver is missing (v. Falkenhausen, 1925), and the liverless frog can even go so far as to build urea from the split-off ammonia (Nonnenbruch and Gottschalk, 1921).

There are, naturally, in connection with this main process, other processes. This is perhaps shown by homogentisic acid formed from the oxidation of the benzene nucleus of tyrosin before or together with the shortening of the side-chain, in the case of the alcaptonuric patient. Homogentisic acid may also be a regular intermediate product in the healthy individual. Another example is the appearance of kynurenic acid in the urine of a rabbit which had been flooded with tryptophane

(Matsuoka and Yoshimatsu, 1925). Also tyrosinase oxidizes free tyrosine to melanin (Raper, 1926, 1927). Thus we see that for the most part the superfluous, free amino acids are shortened and deaminized and their carbon skeleton remainder is utilized.

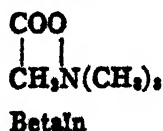
We turn now to a consideration of the metabolism of tissue protein. The protein amines are formed by means of decarboxylation. The carnosin content of the muscles is not affected by the protein content of the food. Carnosin is present in different, but always constant amounts, depending upon the species and kind of muscles (Clifford and Mottram, 1923). Thus, in order that carnosin may be formed directly from food protein, histidyl aspartic acid must be preformed and this must be absorbed in the form of an asparagyl-histidin, a supposition which is highly improbable.



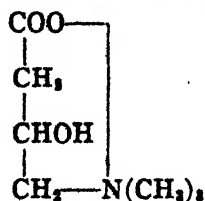
We do not know how creatinine is formed, but we do know that it is not formed from free arginine, even when the arginine is administered subcutaneously, thus avoiding the arginase-rich liver and reaching directly the arginase-free muscles. The small increases in creatinine excretion which Thompson (1917) claims to have obtained are not very convincing.

Epinephrin has never been obtained by the addition of tyrosin to a suprarenal press juice, nor even by the perfusion of the glands themselves. By means of proper stimulation it is possible to extract the already formed and stored epinephrin from the gland and thus weaken the pyro-catechin reaction of the medulla (Anitschkow and collaborators, 1928, 1929).

Other naturally occurring amines have been found in appreciable amounts only in certain organs (hypophysis). This certainly does not indicate that they have been formed through bacterial activity, or that they are merely accidental components of these organs. The fact that the amounts are so small, might be explained by the ease with which they can be oxidized to fatty acids and then further broken down. The betain



content of the common beet did not increase, no matter how much glycine was introduced (Ackerman, 1913).  $\gamma$ -butyric-betaine is regularly found in the urine in the case of phosphorus poisoning as is also carnitine,



Carnitine

the betaine of the  $\gamma$ -amino- $\beta$  hydroxy butyric acid. The mother substance of both of these may be glutamic acid or proline and its respective oxy-acid. Both amino acids on the other hand, when free, are converted to urea.

From such observations I conclude that the breaking down of tissue protein proceeds in a different manner from that of the food protein. Compactly expressed, we can say that in the case of the food protein there is first digestion or hydrolysis and then oxidation or assimilation; in the case of the tissue protein there is first a transformation and limited oxidation and then a separation of the single building stone from the complex protein molecule as in the case of betaine, or of a dipeptid in the case of carnosin, or of a tripeptid in the case of glutathione. An idea of Hofmeister's is that the peptid is to a certain extent a glycine chain and that the organism may obtain such extraordinary amounts of glycine as are found, because the side chains are oxidized away and the remaining glycine chain breaks up.

I have more precisely characterized the idea which Folin evolved, namely, that the exogenous portion of the protein metabolism results in other residues in the urine than the endogenous portion.

As further experimental support of this idea, I have requisitioned cystine. According to Folin the neutral sulphur in the urine is an end product of the endogenous metabolism. Cystine furnished in the diet is oxidized to sulphuric acid, even under the conditions of the nitrogen minimum. In the case of the cystinuric patient it is possible even under these conditions to isolate small amounts of cystine in the urine. Often these traces disappear. They rise and fall with the increase or decrease of the ingested amounts but do not parallel them. The cystine disappears from the urine only, however, on the addition of large doses of sodium bicarbonate, and therefore that portion of the neutral sulphur which is not free cystine, is increased.

Cystin is set free in the case of a healthy person. For the hair continues to grow under the conditions of a protein-free diet and contains, as we may freely assume, according to Abderhalden, its normal cystin content. An interesting and suggestive observation on molting hens has recently been reported by Ackerson, Blish and Mussehl (1926). On a N-free diet molting hens break down tissue protein in order to build up the new feathers. Small amounts of cystin prevented this breakdown to a certain extent.

The cystin metabolism of the liver also continues unchanged. In work of our laboratory, not yet published, Mr. Mukoyama found taurocholic acid unchanged in N starvation experiments covering a long period of time and thus confirmed the results of Whipple. Mukoyama was even able to increase the taurine content of the bile with the aid of cholic acid (very much at the beginning and then less so) and after exhausting in this way the amount of cystin stored, could produce taurocholic acid formation from taurine and cholic acid fed at the level of the N-minimum. From Bergmann's experiments we knew that cystin was the mother substance of taurine but he did not clearly show whether free cystin could be employed for this purpose. It might have been possible that it had at first become a part of the liver protein before it could be drafted for this purpose. We do not need Mukoyama's experiment to convince us of these results. Taurocholic acid may be, therefore, an example of endogenous formation of a free amino acid. The two-fold origin may be indicated in this manner:

Food Protein → cystin which in the presence of cholic acid yields  
                                   ↗ taurine, especially taurocholic acid.

#### Liver Protein

In the case of N-minimum, however, cystin seems to be only locally set free, since none is found in the circulation. None can be held there with the aid of monobrombenzene and thus be brought as far as bromphenyl-



mercapturic acid. It is of no consequence whether brombenzene is administered by mouth and cystin into the intestine or *vice versa* (Kapfhammer, 1921). Cystin cannot be here replaced by any other sulphur-containing compounds (Muldoon, Shiple and Sherwin, 1924).

Intestinal putrefaction on a N minimum diet is greatly depressed through active carbohydrate fermentation. In spite of this, we find no

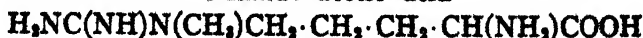


disappearance of the aromatic hydroxy acids in the urine. But, according to Abderhalden (1912), fed tyrosin is completely oxidized even in larger amounts. We have here an indication that tyrosin is not a step in the intermediary metabolism of the tyrosin complex in the tissues. We know the end products of the suprarenals and thyroid glands, epinephrin and thyroxin, and we know from the alcaptonuric that relatively very little homogentisic acid is derived from the endogenous protein metabolism (Katsch, 1918, 1920; Neubauer, 1929). It is possible, as Neubauer thinks, that since little tyrosin is present in such cases, that which is present is used for more important purposes; but it may also be assumed that the tyrosin is separated in a form which is no longer capable of being oxidized to homogentisic acid.

As a third building stone, I should like to introduce arginine. It also yields proofs that it is broken down differently when it comes from tissue protein than when it is fed in excess. Arginine is considered to be the mother substance of creatinine. According to the usual scheme of the breaking down of amino acids, the intermediate steps in its breakdown should be  $\gamma$ -guanidine-butyric acid and guanidine-acetic acid. Or in case the methylation takes place immediately we would have  $\delta$ -methyl-arginine and  $\gamma$ -methyl-guanidine-butyric acid. None of the aforementioned substances, however, yields creatinine in the animal body. The only probable one is guanadine-acetic acid which Jaffe has reported. But one must not conclude from this that it represents a normal intermediary product, for its methylation takes place only to a small extent, although the animal body is capable of methylating other substances foreign to it. Moreover, the guanidine acids are difficultly soluble substances and show an entirely different chemical behavior from arginine. For that reason Kapfhammer, Flaschentraeger and I (1922) developed the synthesis of  $\delta$ -methyl-arginine.



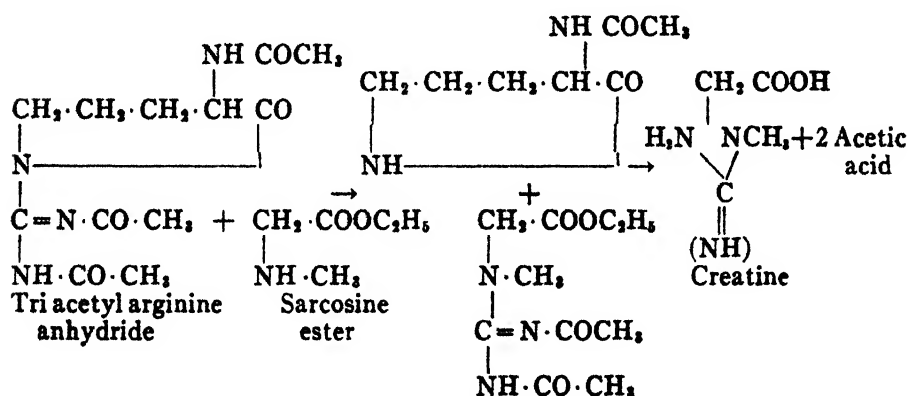
Guanido acetic acid



$\delta$ -Methyl-arginine

The fact that this product also, which differs only so slightly from arginine, gave no creatin, surprised us more than that Thompson was able to prepare creatin from arginine and biological methylation agents. The methyl arginine was not all broken down but was found excreted in the urine completely unchanged. I conclude from this that the arginine behaves similarly to the aromatic amino acids. In their case the moiety

oxidation must take place before the breaking down at the alpha C atom. In the same manner, the arginase must first split the guanidine moiety so that then it is possible for the ornithine to go over into sugar in the liver perhaps by way of  $\gamma$ -amino-butyric acid (Felix and Tomita, 1923; Corley, 1926; Keil, 1927). The methyl group hinders the splitting action of the arginase. The further breaking down in the liver is consequently also depressed and the substance behaves as a foreign body, easily soluble, and is removed by way of the kidneys. But we have flooded the body with the methyl arginine and a portion of it must surely have succeeded in reaching the muscles. There it should, according to the usual hypothetical scheme, have been broken down oxidatively in the absence of arginase. That did not happen, however. I concluded from this that perhaps here, in accordance with the scheme of Hofmeister, only the side chain of the peptid-linked arginine was oxidized and that creatine resulted from the oxidation on the other end.



After this work was done, a period of several years elapsed before the beautiful reports of M. Bergmann (Bergmann and Köster, 1926; Bergmann and Zervas 1927) appeared. In the laboratory it is possible to loosen the guanidine moiety by means of acetylation; its cyanamid complex can then be transferred to other amines, including the ester of glycocoll or of sarcosine. In this manner, Bergmann obtained creatin.

According to Bergmann creatin has two mother substances arginine for the guanidine moiety and the precursors of glycine in the body, which are as yet unknown to us. In the liver, cleavage would take place by means of arginase, but the muscles do not possess this enzyme. Instead, the muscles have available an acylated arginine or creatin, in the form of phosphagen. These substances are constantly being regenerated. One

fact we can accept and that is that muscle arginine probably plays a part in the creatin formation but free arginine never.

In life all chemical reactions are catalyzed by the ferments, which are extraordinarily specific tools and are very definitely dependent upon the structure and configuration of the substrates. They require definite points for attack in order that the necessary intermediary product—the ferment-substrate complex may be formed. In the tissues within the cells we find different conditions, other relationships, other points of attack and other ferments. The cell is also capable of hydrolyzing its protein—that takes place in hunger or when the cell is mechanically destroyed or blood is removed, or in the autolysis of a pneumonic exudate. But the live cell does not have to hydrolyze and probably does not do it so far as that portion which involves the wear and tear quota is concerned. To be consumed means to be used, but not to be lost; if I may so express it. It is spent, after due consideration, for important, inevitably necessary purchases—for materials to support the functioning of one organ in relation to another and also within the cell itself. These materials are so important, but also so unstable, that the body does not rely upon ingestion which is very often purely accidental. Self management also means self aid and therefore the body is forced to prepare these materials under its own direction from its own constitution; that is to say, right there and then from its own protein. We will be able to understand the individual steps in this process when we know more about the synthesis of the body's protein.

K. T.

#### BIBLIOGRAPHY

- Abderhalden, E., 1912, *Zeitschr. f. physiol. Chem.*, LXXVII, 454.  
 Ackermann, D., 1913, *Sitzungsber. med. phys. Soc.*, Würzburg.  
 Ackerson, C. W., M. J. Blish, and F. E. Mussehl, 1926, *Poultry Sci.*, v, 153.  
 Anitschkow, and collaborators, 1928, *Arch. Exp. Path. u. Pharm.*, CXXXVII, 168; 1929, CXL, 235.  
 Benedict, F. G., 1906, *Jour. Biol. Chem.*, I, 263.  
 Berg, W., 1922, 1926, *Pflügers Arch.*, CXC, 543; CCXIV 243.  
 Bergmann, M., and H. Köster, 1926, *Zeitschr. f. physiol. Chem.*, CLIX, 179.  
 Bergmann, M., and L. Zervas, 1927, *ibid.*, CLXXII, 277.  
 Clifford, W. M., and Mottram, V. H., 1928, *Biochem. Jour.*, XXII, 1246.  
 Corley, R. C., 1926, *Jour. Biol. Chem.*, LXX, 99.  
 Dakin, H., 1926, *Zeitschr. f. physiol. Chem.*, CLXVII, 347.  
 Falkenhausen, M. v., and Siwon, P., 1925, *Arch. exp. Path. u. Pharm.*, CVI, 126.  
 Fearon, W. R., 1926, *Physiol. Rev.*, VI, 399.  
 Felix, K., and Tomita, M., 1923, *Zeitschr. f. physiol. Chem.*, CXKXVIII, 40.  
 Grund, G., 1910, *Zeitschr. f. Biol.*, LIV, 173.  
 Kapfhammer, J., 1921, *Zeitschr. f. physiol. Chem.*, CXVI, 302.  
 Katsch, G. D., 1918, 1920, *Arch. f. klin. Med.*, CXXVII, 210, CXXXIV, 59.  
 Keil, W., 1927, *Zeitschr. f. physiol. Chem.*, CLXXII, 310.  
 Lloyd, Dorothy Jordan, 1926, *Chemistry of the proteins*, London.

- Mann, F. C., and Magath, Th. B., 1924, *Ergeb. d. Physiol.*, XXIII, 1 Abt. 212.
- Martin, C. J., and Robison, R., 1922, *Biochem. Jour.*, XVI, 407.
- Matsuoka, Z., and Yoshimatsu, N., 1925, *Zeitschr. physiol. Chem.*, CXLIII, 206.
- Mitchell, H. H., and Hamilton, T. G., 1929, *Biochemistry of the amino acids*, New York.
- Mitchell, H. H., Nevens, W. B., and Kendall, F. E., 1922, *Jour. Biol. Chem.*, LII, 417.
- Montgomery, E. G., 1925, *Biochem. Jour.*, XIX, 71.
- Muldoon, J. A., G. L. Shiple, and C. P. Sherwin, 1924, *Jour. Biol. Chem.*, LIX, 675.
- Murlin, J. R., 1907, *Amer. Jour. Physiol.*, XIX, 285.
- Neubeuer, O., 1929, *Handb. d. norm. u. pathol. Physiol.*, V, 754.
- Neubauer, O., *loc cit.* No. 6, 857.
- Nonnenbruch, W., and Gottschalk, A., 1921, *Arch. exp. Path. u. Pharm.*, XCVI, 115.
- Osborne, T. B., and Mendel, L. B., 1915, *Jour. Biol. Chem.*, XX, 351.
- Raper, H. R., 1926, 1927, *Biochem. Jour.*, XX, 735; XXI, 89.
- Robison, R., 1922, *Biochem. Jour.*, XVI, 111.
- Sherman, H. C., and Campbell, H. L., 1924, *Jour. Biol. Chem.*, LX, 5.
- Thomas, K., 1910, *Arch. f. Physiol.*, Suppl., 249.
- Thomas, K., 1910, not published, *Arch. f. Physiol.*, Suppl., 249.
- Thomas, K., Kapfhammer, J., and Flaschenträger, B., 1922, *Zeitschr. f. Physiol. Chem.*, CXXIV, 75.
- Thompson, W. H., 1917, *Jour. Physiol.*, LI, 111.
- Wieland, H., and Bergel, F., 1924, *Lieb. Ann. Chem.*, CDXXXVIII, 196.
- Zeller, H., 1914, *Arch. f. Physiol.*, 213.



MAY, 1930

THE SIGNIFICANCE OF SURFACE AREA  
DETERMINATIONS

BY H. H. MITCHELL

*(From the Department of Animal Husbandry,  
University of Illinois, Urbana)*

Received for Publication—October 3, 1929

THE selection of methods of measuring the surface area of animals is ordinarily made upon the basis of accuracy and of convenience. An accurate method is considered to be one that will give reproducible results, while it is, of course, obvious that methods adaptable to small animals may be entirely impracticable for large animals. However, whether the surface is determined by measuring the area of the removed hide, or of a mold fitted closely to the body, or by the mechanical integration of the area of the hide *in situ*, or by other means, the prevailing impression seems to be that the surface area of an animal is a definite measurement to which all good methods should approximate, and hence that all good methods are equivalent in the significance of the results obtained.

The area of a removed hide, if it can be spread out on a flat surface, either as removed or divided into a small number of pieces, and if it is not greatly extensible, may be readily measured, and on remeasuring, a satisfactory duplicate result may be obtained. This is true of cattle hides and of sheep hides. If the skin is greatly elastic it is not susceptible to this treatment. It has been the practice in this laboratory, in measuring the area of chicken skins, which are of this description, to stretch them before outlining them on paper. It is felt that this method was the only one capable of giving results even approximately reproducible, while at the same time it was realized that the results thus obtained were not comparable with results obtained on the skins of other animals, on which it is unnecessary to apply tension.

The extensibility of the hide of an animal when removed from the body, as well as the difficulty of making it lie flat upon a smooth surface, has induced many investigators to turn to other methods of determining surface area. Among these may be mentioned especially the making of molds

of one kind or another of the entire body or of a symmetrical part of the body, and the ingenious method of Brody of determining by a mechanical integrator, the area of the hide *in situ*. Such methods have given highly reproducible results and have contributed to the common belief that the surface area of an animal is a constant.

However, it seems obvious, when this proposition is critically examined, that it cannot be strictly true and may in fact be considerably in error. An animal may change its shape considerably by changing the position of its limbs, its head, and its trunk and each change in shape will occasion a change in surface area. The success of methods of determining directly the surface area of animals, and the close reproducibility of the results obtained, must depend upon the fact that a standard position of the body and its appendages is maintained throughout a series of measurements.

In order to ascertain the extent to which change in position will affect surface area, the surface area of a number of chickens and rats was determined by a mold method, described in the following paper, the position of the bodies being varied between two extremes. With the chickens, the accuracy of the method was first tested by cutting the gauze mold from the body in the median sagittal plane, the limbs being in the same position on the right and left side. In another series of trials, the limbs on one side of the body were placed in a position as nearly contracted as the method of putting on the mold permitted, while the limbs on the other side were fully extended. The areas of the two sides were then compared as before.

The results in the first series, in which both legs were contracted and both wings extended, are summarized in Table I.

TABLE I  
THE SURFACE AREA OF THE RIGHT AND LEFT HALVES OF CHICKENS\* WHEN BOTH  
LEGS ARE CONTRACTED AND BOTH WINGS ARE EXTENDED.

Bird No.	Sex	Body weight grams	Surface area of		Per cent difference
			Left side sq. cms.	Right side sq. cms.	
22	pullet	1074	483	491	1.64
24	cockerel	1799	646	659	1.99
25	cockerel	1978	693	707	2.00
26	cockerel	1458	580	580	0.00
27	cockerel	1653	628	625	0.48
28	cockerel	1841	651	641	1.55
29	cockerel	2142	728	729	0.14

\* Exclusive of the shanks and feet and of the combs and wattles.

Evidently the method is capable of close duplication, within 2 per cent. However, if the position of the legs and wings is not symmetrical, the surface areas of the right and left halves may become appreciably different, as the results in Table II indicate.

TABLE II  
THE SURFACE AREA OF THE RIGHT AND LEFT HALVES OF CHICKENS<sup>1</sup> WHEN THE  
LEG AND WING ON ONE SIDE ARE CONTRACTED AND ON THE OTHER EXTENDED

Bird No.	Sex	Body weight grams	Surface of		Per cent difference
			Left side sq. cms.	Right side sq. cms.	
23	pullet	1059	429 <sup>2</sup>	478 <sup>2</sup>	10.81
— <sup>4</sup>	—	—	396 <sup>2</sup>	475 <sup>2</sup>	18.16
24	cockerel	1799	613 <sup>2</sup>	690 <sup>2</sup>	11.82
25	cockerel	1978	698 <sup>2</sup>	686 <sup>2</sup>	1.73
26	cockerel	1458	546 <sup>2</sup>	585 <sup>2</sup>	6.90

<sup>1</sup> Exclusive of the shanks and feet and the comb and wattles.

<sup>2</sup> Leg and wing extended.

<sup>3</sup> Leg and wing contracted.

<sup>4</sup> Results from a second mold of Bird No. 23.

An explanation of the negative result for Bird No. 25 has not been revealed, but with the other birds, particularly the first two, there can be no doubt that the extension of leg and wing has increased the surface area of these appendages over that in the contracted position. However, variable results may be expected unless the extent to which leg or wing is contracted is standardized.

In this experiment with White Leghorn chickens no attempt was made to change the position of head, neck, or trunk. In the work on rats, the position of these members also was varied between two extremes. In the one position, the rat carcass was laid flat on its abdomen and the head and legs were extended to the fullest extent while the mold was being formed. This position is not unlike that sometimes assumed by a rat across the floor of its cage on a very warm day. In the other position, the carcass was placed upon its haunches and made to assume a partially crouched attitude by bending it over a glass feeding cup, tipped on its side. The limbs were partially contracted. Before applying the mold in these positions the hair was removed from the carcasses by immersing them for a few minutes in 10 per cent solution of barium sulfide.



Since the rats used in this study were of different sizes and ages, and since it has not been found satisfactory to take more than one mold of a carcass, because of an apparent shrinkage in the carcass after the first covering is removed, and of a change in the pliability of the skin due to the collodion used as a cement for the gauze covering, the areas obtained in each case have been compared with areas computed from Lee's formula (1)  $S = 10.76W^{0.61} \times 0.310/N_{\text{obs}}$ ,  $N_{\text{obs}}$  being Cowgill's "nutritive correction factor" obtained by dividing the cube root of the weight, in grams, by the length of body from nose to anus, expressed in centimeters. This formula was used in preference to the other formula suggested by Lee, since our rats were evidently in better nutritive condition, with values of  $N_{\text{obs}}$  ranging from 0.288 to 0.304.

In order to discover whether the mold method used in this work was essentially equivalent to Lee's method, by which the depilated skin is fixed, while on the carcass, by dipping into a nitro-cellulose lacquer, two rat carcasses were molded in a position similar to that used by Lee. The first rat, a male, weighed 126 grams, with a body length of 16.5 cms. and an  $N_{\text{obs}}$  of 0.304. The area of the mold was 213 sq. cms. and that predicted from Lee's formula was 210 sq. cms. The second rat, a female, weighed 214 grams and possessed a body length of 19.9 cms. In this case  $N_{\text{obs}} = 0.301$ . The area of the mold was 304 sq. cms., as compared with a predicted area of 293 sq. cms. In the former case, the deviation between observed and calculated areas was 1.46 per cent and in the latter 3.62 per cent. It may be concluded that the two methods are essentially equivalent.

A number of rats were then molded in the two positions described above. The results obtained, and the estimated areas by Lee's formula are summarized and compared in Table III.

It seems clear that the surface area of the rats was markedly affected by the position in which the body was placed. With one exception, the 5 rats molded in the extended prone position gave areas considerably greater than those predicted by the formula of Lee. The average excess for the group was 5.77 per cent. All of the 4 rats molded in the crouched position possessed mold areas considerably less than the predicted areas, averaging -6.20 per cent. The average difference between the areas obtained in the two positions is thus practically 12 per cent. Furthermore, this difference is not due to wrinkling of the skin in the crouched position; it is a result of the change in shape of the carcass and the elasticity of the skin covering it.

1. Lee, M.-O., Determination of the surface area of the white rat with its application to the expression of metabolic results. *Amer. Jour. Physiol.*, 1929, LXXXIX, 24.

The true carcass of the rat is very loosely enclosed in a skin which possesses a high degree of elasticity. This fact is well illustrated by such observations as the following. A male rat weighing 413 grams was skinned, and the area of the skinned carcass was found by the mold method to be 430 sq. cms. The unstretched skin possessed an area of 536 sq. cms. Only

TABLE III  
THE SURFACE AREA OF RATS PLACED IN DIFFERENT POSITIONS

Rat No.	Sex	Body weight grams	Body length cms.	N <sub>obs</sub> <sup>1</sup>	Surface area		Per cent difference
					Observed sq. cms.	Calculated sq. cms.	
Rats in the extended position							
3	f	185	19.8	.288	302	280	+ 7.28
9	m	149	18.1	.293	235	241	- 2.55
11	m	154	18.3	.293	266	246	+ 7.52
6	f	126	17.3	.290	247	220	+10.93
13	f	209	20.3	.293	318	300	+ 5.66
Rats in the crouched position							
7	f	138	17.8	.291	220	232	- 5.45
12	m	202	19.9	.295	271	288	- 6.27
10	m	198	19.6	.297	270	282	- 4.44
8	m	189	19.5	.294	255	277	- 8.63

$$^1N_{obs} = W^{\frac{1}{3}}/L$$

a moderate degree of stretching would increase this area to 630 sq. cms. Similar results were obtained with other rats, so that it appears that the rat is provided with the means of changing its surface area within wide limits by changing the relative position and the shape of its trunk and appendages. That such changes in surface area actually result from change in the shape of the body, the experimental results cited above clearly prove. Even greater changes than those found would be expected if it had been possible to mold the rat carcass in a more contracted position than that chosen.

It may be concluded, therefore, that with chickens and rats, and presumably with other animals also, the surface area is not a definite measurement, but depends to a considerable extent upon the shape of the body, as determined by the position of the body trunk and its appendages. Hence, direct measurements of the surface area of animals placed in some definite position, although they may be closely reproducible and readily conformable to mathematical description, do not possess the definite interpretation ordinarily given them. For example, there is neither a

rational nor an empirical basis for assuming that the basal metabolism of an animal is more closely related to the surface area determined from a certain convenient position of the animal than to the surface area relating to any other position that the animal may naturally assume. And the possible differences between the surface areas of the same animal in different natural positions are not inconsiderable.

These considerations mean that the heat produced by an animal per square meter of body surface possesses no absolute meaning, but that it may still be a highly useful measurement for comparative purposes if the surface measurement for different animals is made by exactly the same method. Within any species it would appear that the basal heat produced per square meter of hide area is just as valuable and significant a measurement as the basal heat produced per square meter of surface area relative to some definite body position, provided the hide area is as accurately determinable as is the surface area. Both measurements can be determinable with accuracy only by the imposition of arbitrary conditions, relating in the one case to the tension, if any, that must be applied to the hide to obtain reproducible results, and in the other case to the position in which the animal must be placed for the most convenient measurement. The hide area of an animal, provided no considerable tension has been applied, may be considered to be a maximum surface area to which the body may attain by change of position. Evidently for animals of different species, the comparability of surface area determinations may be less close, due to the different character of the hide, or to other anatomical differences.

Needless to say, the method of eliminating the effect of differences in size of animal upon basal heat production by expressing the latter per unit of body area is just as valid as ever. Certainly the main value of such expressions is their comparability within the species. In formulas of the Meeh-Rubner type the particular value of the constant becomes thus a matter of indifference, while that of the exponent of the body weight is of first importance.



## THE SURFACE AREA OF SINGLE COMB WHITE LEGHORN CHICKENS

BY H. H. MITCHELL

*(From the Department of Animal Husbandry,  
University of Illinois, Urbana)*

Received for Publication—October 3, 1929

**I**N UNDERTAKING the determination of the surface area of a considerable number of animals by an exacting and time-consuming method, and in burdening the literature with a description of the results secured, one should have a definite conviction of the value and significance of surface area determinations. If the method of expressing basal metabolic rate with reference to a unit of surface area has only its popularity to commend it, as Benedict believes, the time devoted to surface area determinations is hardly time well spent.

The empirical basis of the so-called surface area law seems a sound one, since among a number of animals of the same species differing only in size, the basal heat output, when computed per square meter of body surface, presents a more uniform set of values than when computed per unit of weight. Thus, this method of reference is commonly considered to be the most satisfactory method of eliminating the effect of differences in size of body upon basal heat output. However, this practice carries no implication that no other factor than surface area affects basal heat output. It is a specious argument to urge against it the well known facts that age, sex, muscular development, nutritive condition, and various types of glandular malfunction may also affect basal metabolism; they would disturb any other method of relating basal metabolism to body size.

If a method is empirically sound, it should have some sort of a rational explanation, and not until such an explanation is at hand can the true significance of the method be appreciated and the utmost faith in its validity be felt. Obviously, surface area can be a factor in the determination of basal heat production only in so far as it determines the rate of heat loss from the body, and the necessity of any constant relation between surface area and basal heat production, if such there is, must relate to the necessity of maintaining within narrow limits a constant body temperature. But for warm-blooded animals there is a considerable range of environmental conditions within which the basal heat produced is approximately constant and greater than that needed to maintain body temperature. Hence, within this range heat loss and surface area are not determinants in basal heat output. But within this range, the requisite constancy of pro-

toplasmic temperature is not seriously threatened by environmental conditions. Outside this range, the mechanism for the preservation of a constant cellular temperature is subjected to greater and greater strain. Thus, for environmental temperatures lower than a certain critical value, the rate of heat production of the animal must be increased to keep pace with the increasing heat loss through the surface of the body. Also for environmental temperatures above body temperature, the evaporation of water, mainly from the skin, must be greatly increased, since it alone must bear the burden of heat excretion. Thus, under those environmental conditions threatening the continuation of animal life, the area of the body surface becomes an important factor in the regulation of body temperature.

For warm-blooded animals it may be considered that the basal heat production, in so far as it is related to size of body, is to a very large extent determined by the critical environment. At the critical temperature, the basal heat production must be equal to the minimum heat loss consistent with the maintenance of body temperature. This heat loss will depend upon the surface area of the body, the radiating capacity of the skin and its covering, and the normal body temperature. If all animals possessed the same critical temperature, they must possess the same basal heat production per unit of surface area, except for differences in normal body temperature and in the insulating properties of the skin and its covering. Differences in normal body temperature within the same species of warm-blooded animal are insignificant in this connection, and even among different species they amount to only a few degrees centigrade. Differences in the insulating properties of the skin and its covering may be great, but the more effective the skin is as an insulator of heat, the lower is the critical temperature of the animal, and hence the lower is the environmental temperature to which the basal heat production is adjusted. The net result will be a marked tendency, such as has been observed experimentally, for all animals, regardless of differences with respect to skin covering, body temperature, and critical temperature, to possess approximately the same adult basal heat production per unit of surface area. The effects of age, sex, muscular development, nutritive condition, and glandular functioning on basal metabolism are, of course, unrelated to size and surface area, and their existence does not vitiate, though it may obscure, the relation of surface area to the basal metabolic rate.

Thus, the characteristic features of warm-bloodedness in animals as they have been observed and reported, render inevitable a close relation between surface area and basal heat output. The relation possesses a

physical significance which is entirely ignored in Brody's recommendation (1) that basal metabolism be considered merely as a power function of body weight rather than as a function of body surface.

With the conviction that surface area is a real determinant in basal heat output, the surface areas of 25 Single Comb White Leghorn chickens, varying in body weight from 109 to 2142 grams, were measured in order to derive a formula by which surface area can be readily computed.

The birds were killed by bleeding and debraining and were then dry picked. They were then measured and laid out in a standard supine position with neck and wings extended and legs as nearly contracted as the method of molding permitted. The wings were pinned down in the desired position and the legs were supported on strings suspended from a laboratory ring stand. The comb and wattles were then cut off as were the ear lobes in the larger birds. The surface of the bird was then covered closely with strips of ordinary medical sterilized gauze, either 2 inches or 1 inch in width, which were made to adhere to the body and to each other as they were put in place by collodion applied with a brush. By varying the size of gauze and the length of the strip it was possible to cover all parts of the body regardless of their curvature. However, the shanks and feet were not covered. After the ventral part of the body was covered, the bird was turned over and covered on the dorsal side without changing the position of legs and wings. The completed mold was dry in one hour or less of standing, during which time a slight contraction of the gauze occurred, insuring a tight fit. In removing the mold from the body, it was first cut in two parts along the median sagittal line, and then was cut along the neck, wings, and legs as was found necessary for convenient removal. After removal from the body, the mold was cut up into pieces of such size and shape that they would lie flat, outlined with a pencil on a large sheet of paper, and their combined area determined with the planimeter. Depending upon the size of bird, it was found necessary to cut the mold into 17 to 50 pieces. The comb and wattles were also outlined and the area doubled, allowance being made in the case of the comb for the area of the surface of attachment to the head. The ear lobes, when large enough to require separate treatment, were outlined and measured, and allowance also made for the area of attachment. The area of the shanks and feet was determined by skinning one shank and foot, determining the area by cutting up, outlining, and applying the planimeter, and doubling this area.

Besides the live weight and surface area, three linear measurements were taken, *i.e.*, (a) the over-all length, from tail to tip of beak, (b) the rump-to-shoulder length, and (c) the circumference of thorax taken over the keel

and just behind the wings. On many of the birds, the picked, bled weight was also recorded. These measurements and weights are all contained in Table I.

TABLE I  
THE BODY WEIGHTS, BODY MEASUREMENTS, AND SURFACE AREAS OF WHITE  
LEGHORN CHICKENS

Bird No.	Body weight grams	Surface area sq. cms.	Length over all cms.	Rump to shoulder cms.	Circumference cms.	Picked weight grams
8	110	227	18.0	7.4	11.0	—
9	109	220	18.0	7.4	10.5	—
11♂	235	376	25.0	9.1	14.0	—
12♂	341	526	27.5	11.1	15.0	—
13♂	449	618	29.0	11.8	17.0	—
14♂	555	731	33.0	13.2	18.0	490
15♂	578	781	33.5	13.2	19.5	504
16♀	668	795	35.5	13.7	19.5	570
21♀	840	908	39.5	16.1	22.5	712
18♀	984	1014	39.5	17.0	23.5	861
23♀	1059	1038	42.0	16.5	24.0	920
17♂	1072	1155	40.5	16.7	23.5	948
22♀	1074	1127	44.5	16.8	24.5	937
20♀	1109	1174	41.0	16.9	23.5	947
19♀	1213	1152	41.5	17.6	25.0	1095
5♀	1273	1172	40.5	16.0	24.5	1121
7♀	1329	1247	45.0	17.2	25.0	—
26♂	1458	1470	45.5	18.5	26.5	1270
6♀	1495	1469	46.0	—	29.5	—
10♀	1513	1435	47.0	18.2	27.0	—
27♂	1653	1602	48.0	18.9	28.5	1423
24♂	1799	1684	48.0	19.4	27.5	1612
28♂	1841	1612	48.0	18.0	28.0	1600
25♂	1978	1720	50.5	19.9	29.0	1725
29♂	2142	1894	49.5	21.3	30.0	1918

In attempting to fit a prediction formula to these measurements of surface area, it was realized that a close fit was hardly to be expected, because of a variable feather coat, which would affect body weight, but not body surface as measured from the picked carcass, and because of a variable growth of comb and wattles, depending in particular upon sex and to some extent upon nutritive condition. An extensive growth of comb and wattles would increase the body weight somewhat, but would have an entirely disproportionate effect upon surface area.

Using the method of least squares, the Meeh formula

$$S = kW^{.667}$$

was fitted to the data in Table I, with the result that  $k$  was evaluated at 10.64. The calculated areas of the birds by means of this are given in column 3 of Table II, and the percentage deviations from the observed values in column 4. The average percentage deviation, disregarding signs, is 4.51.

TABLE II  
A COMPARISON OF CALCULATED AND OBSERVED SURFACE AREAS

Bird No.	Observed surface area sq. cms.	$S = 10.64 W^{.667}$ sq. cms.	Per cent difference	$S = 8.19 W^{.706}$ sq. cms.	Per cent difference	$\frac{W^{.333}}{L}$
8	227	244	+ 7.49	226	-0.44	.266
9	220	243	+10.45	224	+1.82	.265
11♂	376	405	+ 7.71	385	+2.39	.247
12♂	526	519	- 1.33	501	-4.75	.254
13♂	618	624	+ 0.97	608	-1.62	.264
14♂	731	718	- 1.78	707	-3.28	.249
15♂	781	738	- 5.51	727	-6.91	.249
16♀	795	813	+ 2.26	805	+1.26	.246
21♀	908	947	+ 4.30	946	+4.18	.239
18♀	1014	1052	+ 3.75	1058	+4.33	.252
23♀	1038	1105	+ 6.45	1115	+7.42	.243
17♂	1155	1114	- 3.55	1124	-2.68	.253
22♀	1127	1115	- 1.06	1126	-0.09	.230
20♀	1174	1140	- 2.90	1151	-1.96	.252
19♀	1152	1210	+ 5.03	1227	+6.51	.257
5♀	1172	1249	+ 6.57	1269	+8.28	.268
7♀	1247	1286	+ 3.13	1308	+4.89	.244
26♂	1470	1368	- 6.94	1396	-5.03	.249
6♀	1469	1391	- 5.31	1421	-3.27	.249
10♀	1435	1402	- 2.30	1433	-0.14	.244
27♂	1602	1487	- 7.18	1526	-4.74	.246
24♂	1684	1573	- 6.59	1620	-3.80	.253
28♂	1612	1598	- 0.87	1646	+2.11	.255
25♂	1720	1676	- 2.56	1732	+0.70	.249
29♂	1894	1767	- 6.71	1832	-3.27	.260
Average			4.51		3.73	.251

If the exponent of  $W$  (body weight in grams) in the Meeh formula, as well as its coefficient  $k$ , are evaluated from the data by the method of least squares, the prediction formula becomes

$$S = 8.19W^{.706}$$



The calculated areas of the birds by this formula, and the percentage deviations, are given in columns 5 and 6 of Table II. The average percentage deviation is 3.73, somewhat less than that obtained with the first formula, and the fit to the data is appreciably better at the two ends of the range. The second formula is thus a distinct improvement over the first. Of the 25 cases only 5 show deviations greater than 5 per cent, and all are within 10 per cent. Closer fits of prediction formulas to surface area measurements have been obtained with other animals, but, as already explained, the prospects of obtaining a close fit of any formula to surface area measurements in chickens are not encouraging.

An attempt was made to improve the formula by the introduction of a term defining the nutritive condition of the animal. According to Cowgill and Drabkin (2), a term that should serve this purpose is obtained by dividing the cube root of the body weight by the body length. In the last column of Table II, this factor, involving the length in centimeters from tail to tip of beak, is given for each bird. If this factor is capable of serving a useful purpose in improving a prediction formula involving only the body weight, it would be expected that, for birds whose calculated areas deviated most widely from the observed, the nutritive correction factor would also be out of line. But a comparison of the last 2 columns in Table II does not reveal such a situation. It is true that the greatest positive deviation, 8.28 per cent, is associated with the highest nutritive correction factor, 0.268, but the next highest factor, 0.266, is obtained with a bird, No. 8, for which a very close prediction of surface area was obtained; this is also true of the next two highest factors, 0.265 and 0.264. The lowest nutritive factor, 0.230, is also associated with a bird for which a very good prediction was secured. These considerations do not indicate that the cause of poor predictions by the use of the second prediction formula was a variable nutritive condition of the birds. Hence, no systematic attempt was made to introduce this factor into the prediction formula.

From the fact that the six largest positive percentage deviations of predicted from observed areas relate to females, while the five largest negative deviations relate to males, it seems evident that sex is a determining factor in surface area, even before excessive comb growth is present (Nos. 12 and 15). Unfortunately, the present data are not suitable for the derivation of separate prediction formulas for each sex, since the females measured are all of intermediate weight, while the males are, with two exceptions, either lighter than 578 grams or heavier than 1653 grams.

## SUMMARY

The surface area of Single Comb White Leghorn chickens of both sexes may be predicted by the equation

$$S = 8.19 W^{.708}$$

$S$  being the surface area in square centimeters and  $W$  the body weight in grams. This formula is a distinct improvement over the Meeh formula, and apparently cannot be improved by the introduction of a factor defining nutritive condition. Except for the distribution by weight of the pullets and cockerels measured in this experiment, it would be profitable to devise separate prediction formulas for each sex.

## REFERENCES

1. Brody, S., Comfort, J. E., and Mathews, J. S., Further investigations on surface area with special reference to its significance in energy metabolism. *Missouri Agr. Exp. Sta. Res. Bul.* No. 115, 1928.
2. Cowgill, G. R., and Drabkin, D. L., Determination of a formula for the surface area of the dog together with a consideration of formulae available for other species. *Amer. Jour. Physiol.*, 1927, LXXXI, 36.





## A COMPARATIVE STUDY OF LIQUID AND DRY MILK AS ANEMIA-PRODUCING DIETS

By

G. C. SUPPLEE, O. D. DOW, G. E. FLANIGAN, AND O. J. KAHLENBERG

*(From The Research Laboratories of The Dry Milk Company,  
Bainbridge, New York.)*

Received for Publication—October 7, 1929

VARIOUS researches have directed attention to the existence of minute amounts of certain metals in the animal organism. Body tissue and fluids, and numerous dietary substances, have been subjected to analytical survey for the purpose of explaining the significance of these small amounts of metal, but with the exception of iron and possibly copper, little progress has been made in determining their physiological function. Milk as a body secretion and as a food substance has claimed the attention of numerous investigators interested in determining basic relationships between the elemental composition of this product and the complete dietary. The normal iron content of milk has been quite definitely determined and within recent years the normal copper content of milk appears to have been fairly well established (1-5). Recent investigations dealing with the causes of nutritional anemia of experimental animals fed on an exclusive milk diet have centered attention on these two elements (6-12). The general conclusions from these investigations, either stated or implied, are to the effect that the anemia produced by a prolonged milk diet is due primarily to the low iron and copper content of the milk. The specificity of copper in preventing or curing nutritional anemia is not wholly conceded, however, by Elden and collaborators (13) or by Drabkin and Waggoner (14).

The apparent importance of the iron and copper content of milk as factors determining its adaptability for certain dietaries, has brought into relief the significance of numerous observations made at this laboratory during several years of study of the factors of similarity and dissimilarity between natural fluid milk and dry milk as prepared by the revolving cylinder process. Unpublished data obtained in 1919 revealed that desiccated milk prepared by this method normally contains from 2 to 5 times more iron than the natural liquid milk. This increase in iron content is obviously due to the intimate contact of the milk with the desiccating cylinders. Attempts to segregate this additional iron increment as an inorganic substance have failed thus far. There is reason to believe that it may exist in the dry milk as an organic combination.

The desiccating operation is known to affect certain constituents of the milk, particularly the proteins. Numerous clinical observations (15) seem to indicate that this alteration has a favorable effect upon the assimilability of the product. It is conceivable, therefore, that the assimilation of any existing iron compounds in the dry milk, particularly if associated with the protein material, may likewise be favorably affected.

Other investigations, particularly those in reference to the keeping quality of dry milk (16), led to the discovery that under certain conditions minute amounts of copper existing as an impurity in the desiccating cylinders, might be added to the dry product during the desiccating operation. Analyses of the metal of the drying cylinders revealed a copper content varying between 0.032 and 0.185 per cent. Milk dried on cylinders which were relatively new leached out the soluble copper to a variable degree, causing an increase in the copper content of the dry milk (calculated to the original fluid milk basis) of from 0.04 to 0.37 parts per million. Older drying cylinders, which had been subjected to the continual solvent action of the milk daily for a period of years, did not give up measurable amounts of copper to the milk dried on them.

Studies designed to determine whether manganese was imparted to the dry milk during the desiccating operation have also been made. Analyses of the metal of the cylinders have revealed a fairly constant manganese content varying from 0.11 to 0.17 per cent. Manganese has not been found in the dry milk, either by the usual analytical methods or by spectroscopic analysis.

In addition to these various analytical results, dry milk prepared by the roller process, used for various feeding experiments, has failed to cause the degree of nutritional anemia commonly reported for natural fluid milk. These general observations, accruing over a period of several years, taken together with clinical reports which have occasionally come to our attention indicating a degree of improvement in certain anemic conditions and emaciations when this dry milk was used, have prompted a systematic and comparative study of this type of dry milk in reference to the anemia problem.

#### EXPERIMENTAL

##### *Preliminary Data*

Since control measures for preventing extraneous copper contamination in the milk handled at the particular factory furnishing the product for the following experiments have been in operation for several years, abundant data were available showing the normal copper content of the liquid

and dry product. These data show that the copper in the dry milk when calculated to the liquid basis is substantially the same as for natural fluid milk, namely, within the limits of 0.50 to 0.75 milligrams per liter.<sup>1</sup> A few comparative analyses for iron confirmed previous data showing an increase in iron content in the dry milk to three or four fold greater than that in the liquid milk from which it was prepared.

Although preliminary feeding tests were started prior to the recently published investigations indicating the importance of the copper constituent, the analytical data from the test samples permit an accurate record of both the copper and iron content of the liquid and dry milk used in the preliminary work, the plan of which follows:

A few days prior to the birth of the young rats, several mothers were isolated in individual cages with access to the usual stock ration consisting of corn meal 76 parts; linseed meal 16 parts; crude casein 5 parts; alfalfa meal 2 parts; sodium chloride one-half part and calcium carbonate one-half part. Within 4 days after the birth of the litters the stock feed and water were taken from the mothers and the milk diets supplied fresh each day thereafter. One group received fresh liquid milk. The second group received the desiccated product, reconstituted to the original fluid milk basis with distilled water, to furnish the same content of milk solids per unit of volume as was furnished in the natural fluid milk. The third group received the same desiccated milk as the second group, but in this instance the dry product was reconstituted to give a mixture containing 30 per cent solids or approximately 3 times the milk solids per unit of volume received by the other two groups.

As the young of each litter reached the weaning age they were placed in individual cages of lacquered galvanized wire cloth and continued on the respective diets received by the mothers during the suckling period. Beginning at 30 days of age the food consumption for each individual rat was recorded. At 30, 60, 90 and 120 days of age 4 to 6 rats receiving the respective diets were killed and certain analytical determinations made as shown

<sup>1</sup> A recent report by Elvehjem, Steenbock and Hart (17) states that the normal copper content of milk is materially lower than these figures. Their results were obtained by a method wherein the copper was precipitated as the sulphide. According to experiences in this laboratory, the precipitation of such minute amounts of copper as are found in milk by hydrogen sulphide cannot be relied upon as quantitatively accurate. The results as obtained in this laboratory by hydrogen sulphide precipitation methods have been too low, as has been readily determined by appropriate check analyses and corroborative evidence from solubility tables. Various check analyses, wherein reagents, apparatus and other important checks on the manipulations, which have been made at intervals during the past 10 years have indicated the Xanthate method as suitable for determining copper in milk.

TABLE I  
AVERAGE ANALYSES OF WHITE RATS MAINTAINED ON STOCK COLONY RATION, LIQUID MILK AND RECONSTITUTED DRY MILK

Ration and age at time of analysis	Weight of rat (gms)	Dry matter (gms)	% Dry matter	% Ash on dry basis	Copper on dry basis (p.p.m.) <sup>1</sup>	Iron on dry basis (p.p.m.) <sup>2</sup>	Ratio Copper to Iron (dry basis)	Blood Count (Millions)
Stock colony ration, grain mixture and whole milk <i>ad lib.</i>	5.45	0.85	15.73	10.97	22.0	315.4	1:14.3	
	10.33	2.00	19.35	11.08	16.0			
	46.09	12.88	27.94	9.97	9.7	119.3	1:12.3	7.95
	127.91	38.99	30.49	10.89	4.5	117.8	1:26.1	9.73
	184.80	59.18	32.02	11.61	4.6	96.5	1:20.9	9.33
120 days	211.50	72.40	34.23	11.54	4.3	100.8	1:23.4	11.01
Liquid milk only	23.89	6.10	25.52	14.97	27.6	132.7	1:4.8	7.75
	46.92	12.82	27.33	17.71	11.5	68.0	1:5.9	4.55
	80.91	26.75	33.06	13.09	5.8	53.2	1:9.1	7.34
	73.20	20.75	28.34	19.42	11.7	69.1	1:5.9	5.93
Dry milk reconstituted to original liquid milk basis	29.75	7.21	24.23	13.29	22.8	166.8	1:7.3	8.25
	79.40	22.80	28.71	15.36	7.2	108.2	1:15.0	11.31
	117.00	39.66	33.89	13.31	3.1	98.8	1:31.9	11.38
	149.00	52.00	34.90	12.05	2.6	78.9	1:30.3	12.04
Dry milk reconstituted to basis of 30% solids	39.96	10.59	26.50	12.78	21.3	82.0	1:3.8	6.50
	88.16	27.00	30.62	12.99	9.6	87.0	1:9.0	12.19
	128.00	40.40	31.56	13.94	5.2	93.4	1:18.0	11.16
	153.00	51.92	33.93	12.86	3.0	67.6	1:22.5	8.59

in Table I. For the purpose of obtaining similar data to represent standard conditions prevailing in the stock colony, other groups of young animals receiving the stock ration were isolated in individual cages and analytical data were obtained at 30 day intervals as also shown in Table I. During the observation period from the 30th to the 120th day, aliquot samples of the test milks and stock ration were taken and subsequently analyzed for iron and copper. These data with the daily or period food consumption permitted calculation of the copper and iron intake for each of the 30 day intervals. These results are shown in Table II in which is also given the average amount of copper and iron retained by the body during each 30 day period.

The results given in Tables I and II show that white rats fed on the reconstituted dry milk diet exclusively, grew better than those fed natural fluid milk. All animals receiving the desiccated product were raised to maturity. Blood counts were within normal limits and the apparent physical condition of the animals was good, although weight at corresponding ages was not so great as for those animals receiving the stock ration. Among the animals receiving the natural fluid milk diet, a number of deaths resulted, particularly between the ages of 30 to 60 days. The animals receiving this diet were emaciated and anemic, as indicated by physical condition, color of skin and eyes, and according to blood counts.

It is to be noted that the animals one day old contained a larger amount of copper per unit of dry substance than at later periods of life. This confirms the observations of Bodansky (18) that copper as well as iron is relatively high in the new born animal organism. At 30 days of age there was a greater amount of copper in the bodies of the rats receiving the milk diets than in those receiving the stock ration. The iron content of the animals receiving the milk diets was lower than of those receiving the stock ration. In the case of those receiving the natural fluid milk, the iron content of equivalent weights of dry body substance after correction for difference in growth was only about 46 per cent of that contained in the bodies of the animals receiving the stock ration; those receiving the reconstituted dry milk made up to the original liquid milk basis had about 80 per cent of the iron of the animals receiving the stock ration; and those receiving the reconstituted dry milk containing 30 per cent solids had about 57 per cent of the iron of those receiving the stock ration. At this stage of development the blood count for each group was substantially the same and obvious manifestations of anemia had not developed in any of the animals receiving the milk diets. It is apparent, however, that differences in the metabolism involving iron and copper were taking place in the body when the animals were 30 days old.



TABLE II  
AVERAGE COPPER AND IRON INTAKE AND RETENTION BY WHITE RATS MAINTAINED ON STOCK COLONY RATION, LIQUID MILK  
AND RECONSTITUTED DRY MILK

Ration and Observation period	Food Consumed (gms. dry basis)	Iron Intake (mgs)	Iron gained or lost %		Copper Intake (mgs)	Copper gained or lost %	
			(mgs)			(mgs)	
Stock colony ration 30 to 60 day period...	201	6.77	3.06+	45.2+	3.06	.051+	1.66+
60 to 90 day period.....	610	11.36	2.64+	23.2+	3.20	.096+	3.00+
90 to 120 day period.....	1218	19.38	1.59+	8.2+	6.55	.040+	0.61+
Total for 30 to 120 day period.....	2029	37.51	7.29+	19.4+	12.81	.187+	1.46+
Liquid Milk only 30 to 60 day period.....	100.3	2.50	0.07+	2.8+	0.68	0.03—	
60 to 90 day period.....	188.8	4.72	0.55+	11.6+	1.28	0.008+	0.62+
90 to 120 day period.....	24.2	0.61	0.01+	1.6+	0.17	0.08+	47.0+
Total for 30 to 120 day period.....	313.3	7.83	0.63+	8.0+	2.13	0.058+	2.7+
Dry milk reconstituted to original liquid milk basis 30 to 60 day period.....	125.8	2.18	1.26+	57.8+	1.08	0.00	
60 to 90 day period.....	281.2	4.90	1.45+	29.6+	1.94	0.04—	
90 to 120 day period.....	239.0	4.16	0.91+	4.5+	2.53	0.013+	0.51+
Total for 30 to 120 day period.....	646.0	11.24	2.90+	25.8+	5.55	0.027—	
Dry milk reconstituted to basis of 30% solids. 30 to 60 day period.....	232	5.26	1.48+	28.1+	2.48	0.034+	1.36+
60 to 90 day period.....	276	6.27	1.43+	22.8+	2.95	0.049—	
90 to 120 day period.....	253	5.74	0.26—		2.71	0.055—	
Total for 30 to 120 day period.....	761	17.27	2.65+	15.3+	8.14	0.070—	

The value of these preliminary comparisons may be briefly summarized as indicating the importance of an increased amount of available iron in the milk diet as a preventive for the characteristic milk anemia in white rats. This appears to be accomplished, in part at least, by the additional iron taken up by this type of dry milk during the desiccating process. From the foregoing data it appears that the copper content of the milk used for these comparisons may be adequate, quantitatively considered, for maintaining a substantial degree of normality of blood formation in the white rat, providing other conditions are supplied. Since the milk solids consumed per rat as reconstituted dry milk were greater than the milk solids consumed in the form of natural liquid milk, a greater copper and iron intake naturally resulted. The results at least clearly indicate the significance of an increased iron content and suggest probable importance of the assimilability of the inorganic as well as the organic constituents. Miller, Forbes and Smythe (19) have already called attention to the differences in assimilability of the iron of various protein foods.

#### FURTHER COMPARISON OF THE MILK DIETS

In order to study further the cause of the apparent differences in anemia-producing properties of liquid milk and the desiccated milk, a more detailed plan of investigation was undertaken.

Young rats 30 days old were isolated in carefully lacquered individual cages and various milk rations, as shown by the accompanying charts, were fed for periods up to 14 weeks. Weight, blood counts, and hemoglobin, determined with the Dare Hemometer, were taken weekly. The various milks, both liquid and dry used for the comparative study were obtained in parallel daily from a commercial milk drying plant. Slight variations in inorganic composition other than those caused by the drying operation itself were therefore avoided. All reconstituted dry milk was made up to the original fluid milk basis with distilled water. For those groups which required a supplement of milk ash, the particular sample was ashed daily and fed with the milk on the following day. Charts I to XIV have been prepared from the average of the results obtained from four rats for each group receiving the different rations. Table III shows the average food consumption and copper and iron intake per rat per week.

In order to ascertain whether minute qualitative differences in the inorganic constituents of the liquid and dry milk might account for the difference in anemia-producing results of the two products, the ash of a single sample of dry milk and the ash from the liquid milk from which it was

TABLE III  
AVERAGE FOOD, COPPER AND IRON INTAKE PER RAT PER WEEK DURING OBSERVATION PERIOD

Group	Food, copper and iron intake	Weeks of Observation													
		1st.	2nd.	3rd.	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th
I—Continuous feeding liquid whole milk	Milk (cc)	227	310	313	322	298	306	283	262	277	269				
	Cu (mgs)	0.14	0.19	0.20	0.21	0.19	0.19	0.18	0.16	0.17	0.17				
	Fe (mgs)	0.30	0.41	0.42	0.43	0.40	0.41	0.38	0.35	0.37	0.36				
II—Continuous feeding, re-constituted dry milk from cylinders polished daily	Milk (cc)	271	420	518	508	563	560	570	623	647	650	684	653	669	683
	Cu (mgs)	0.16	0.25	0.30	0.30	0.33	0.33	0.34	0.37	0.38	0.38	0.40	0.38	0.39	0.40
	Fe (mgs)	0.68	1.05	1.30	1.27	1.41	1.41	1.43	1.56	1.63	1.63	1.72	1.63	1.68	1.72
III—Continuous feeding, re-constituted dry milk from cylinders not polished	Milk (cc)	266	353	449	505	552	588	575	563	650	692	761	748	743	704
	Cu (mgs)	0.14	0.19	0.24	0.27	0.30	0.32	0.31	0.30	0.35	0.38	0.41	0.41	0.40	0.38
	Fe (mgs)	0.53	0.70	0.89	1.01	1.10	1.17	1.15	1.12	1.30	1.38	1.52	1.49	1.48	1.40
IV—Continuous feeding, liquid milk supplemented with liquid milk ash	Milk (cc)	313	430	492	534	486	478	432	433	495	530	565			
	Cu (mgs)	0.36	0.50	0.57	0.62	0.56	0.55	0.50	0.50	0.57	0.62	0.65			
	Fe (mgs)	0.64	0.88	1.01	1.10	1.00	0.98	0.88	0.88	1.01	1.10	1.16			
V—Continuous feeding, liquid milk supplemented with ash of dry milk from cylinders polished daily	Milk (cc)	270	423	476	513	507	516	448	640	612	658	632			
	Cu (mgs)	0.30	0.47	0.53	0.57	0.56	0.57	0.49	0.71	0.68	0.73	0.70			
	Fe (mgs)	0.83	1.29	1.46	1.56	1.54	1.56	1.37	1.96	1.87	2.01	1.90			
VI—Continuous feeding, liquid milk supplement with ash of dry milk from cylinders not polished	Milk (cc)	269	402	427	421	455	451	473	446	501	542	487			
	Cu (mgs)	0.29	0.43	0.46	0.45	0.49	0.49	0.51	0.48	0.54	0.58	0.52			
	Fe (mgs)	0.82	1.07	1.14	1.12	1.22	1.21	1.26	1.19	1.34	1.45	1.30			
VII—Fed liquid milk, subsequent change* to reconstituted dry milk from cylinders polished daily	Milk (cc)	252	412	420	430	432	460*	395	463	530	561	605	639	640	644
	Cu (mgs)	0.16	0.27	0.28	0.28	0.28	0.27*	0.23	0.27	0.31	0.33	0.36	0.38	0.38	0.38
	Fe (mgs)	0.29	0.49	0.49	0.50	0.50	1.15*	0.99	1.16	1.33	1.41	1.52	1.61	1.61	1.62

TABLE III (continued)

Group	Food, copper and iron intake	Weeks of Observation													
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th
VIII—Fed liquid milk, subsequent change* to reconstituted dry milk from cylinders not polished	Milk (cc)	319	465	503	490	453*	485	529	571	669	693	743	750	751	770
	Cu (mgs)	0.21	0.30	0.33	0.32	0.30*	0.26	0.29	0.31	0.36	0.38	0.40	0.41	0.41	0.42
	Fe (mgs)	0.37	0.54	0.59	0.57	0.53*	0.97	1.05	1.14	1.33	1.38	1.48	1.50	1.50	1.54
IX—Continuous feeding, liquid milk, subsequent supplement* of ash from liquid milk	Milk (cc)	304	451	482	458*	440	447	429	374	428	541	558			
	Cu (mgs)	0.20	0.30	0.32	0.52*	0.51	0.51	0.49	0.42	0.49	0.61	0.65			
	Fe (mgs)	0.36	0.53	0.56	0.92*	0.90	0.90	0.87	0.77	0.87	1.10	1.13			
X—Continuous feeding, liquid milk, subsequent supplement* of ash of dry milk from cylinders polished daily	Milk (cc)	288	451	535	529	496	487*	458	718	660	748	717	727		
	Cu (mgs)	0.19	0.30	0.35	0.35	0.33	0.32*	0.51	0.79	0.73	0.83	0.79	0.80		
	Fe (mgs)	0.34	0.53	0.63	0.62	0.58	0.57*	1.40	2.20	2.02	2.29	2.20	2.22		
XI—Continuous feeding, liquid milk boiled for 2 minutes	Milk (cc)	327	481	527	503	460	490	454	488						
	Cu (mgs)	0.19	0.27	0.30	0.29	0.26	0.28	0.26	0.28						
	Fe (mgs)	0.43	0.63	0.69	0.65	0.60	0.64	0.59	0.64						
XII—Continuous feeding, reconstituted spray process dry milk	Milk (cc)	267	448	468	398	420	437	405	421	458	489	515	524		
	Cu (mgs)	0.17	0.28	0.30	0.25	0.27	0.28	0.25	0.27	0.29	0.31	0.33	0.33		
	Fe (mgs)	0.32	0.55	0.57	0.49	0.51	0.53	0.49	0.51	0.56	0.60	0.63	0.64		
XIII—Cont. feeding, reconstituted dry milk prepared from reconstituted spray process dry milk as used for Group XII	Milk (cc)	288	580	591	637	691	736	708	717	775	783				
	Cu (mgs)	0.28	0.37	0.38	0.40	0.44	0.47	0.45	0.46	0.49	0.50				
	Fe (mgs)	1.26	2.53	2.58	2.78	3.01	3.36	3.09	3.13	3.38	3.42				
XIV—Regular stock colony ration	Grain mixture (gms)	43	62	66	61	62	58	54	67	52	59	48	46	43	
	Milk (cc)	89	107	142	126	133	158	165	166	173	165	185	180	210	
	Cu (mgs)	0.47	0.67	0.72	0.67	0.68	0.65	0.62	0.75	0.67	0.67	0.57	0.55	0.53	
	Fe (mgs)	3.44	4.93	5.26	4.87	4.97	4.68	4.37	5.38	4.24	4.76	3.94	3.78	3.49	

made were subjected to spectroscopic examination<sup>2</sup>. No qualitative differences could be detected by this method of examination. The ash from a composite sample of dry milk accumulated over a period of several months likewise failed to show a qualitative constitution different from that of the ash of liquid milk. The spectrograms of these samples are shown in Plate A.

#### SUMMARY

1. The production of nutritional anemia in white rats fed exclusively on a natural fluid milk diet is confirmed. A similar anemic condition also resulted from the feeding of liquid milk boiled for two minutes, and from the feeding of reconstituted spray process milk powder containing normal amounts of iron and copper.

2. Reconstituted dry milk having the same copper content as the fluid milk from which it was prepared, but with an increased iron content resulting from contact of the milk with the drying cylinders, prevented the development of the characteristic milk anemia during the observation periods herein recorded. This milk also corrected to an appreciable degree the anemic condition resulting from the prolonged natural fluid milk diet. Immediate response to the change to the dry milk diet was shown by improved physical condition of the animals, increased blood count and ascending hemoglobin content.

3. In cases where the iron content of the reconstituted milk was approximately twice the iron content of fluid milk, the blood count and hemoglobin did not reach the levels attained by the feeding of a normal stock ration. In one group where the iron content of the reconstituted milk was approximately four times that of normal milk, a normal hemoglobin level and normal blood count were maintained throughout the observation period.

4. Increasing the iron and copper content of fluid milk as inorganic additions furnished through the medium of liquid milk ash and dry milk ash did not furnish an appreciable degree of protection against anemia, nor so great a degree of protection as did the feeding of the unsupplemented reconstituted milk.

4. The results are not conclusive in showing that the copper content of the milk is always the vital factor concerned in the anemia of white rats receiving milk diets exclusively.

5. The increased quantity of iron in the desiccated milk and such other changes in its chemical structure as may concurrently result from the desiccating operation, appear to impart measurable anti-anemic properties to this type of milk.

<sup>2</sup> The spectroscopic examinations were made by Dr. Jacob Papish at Cornell University.

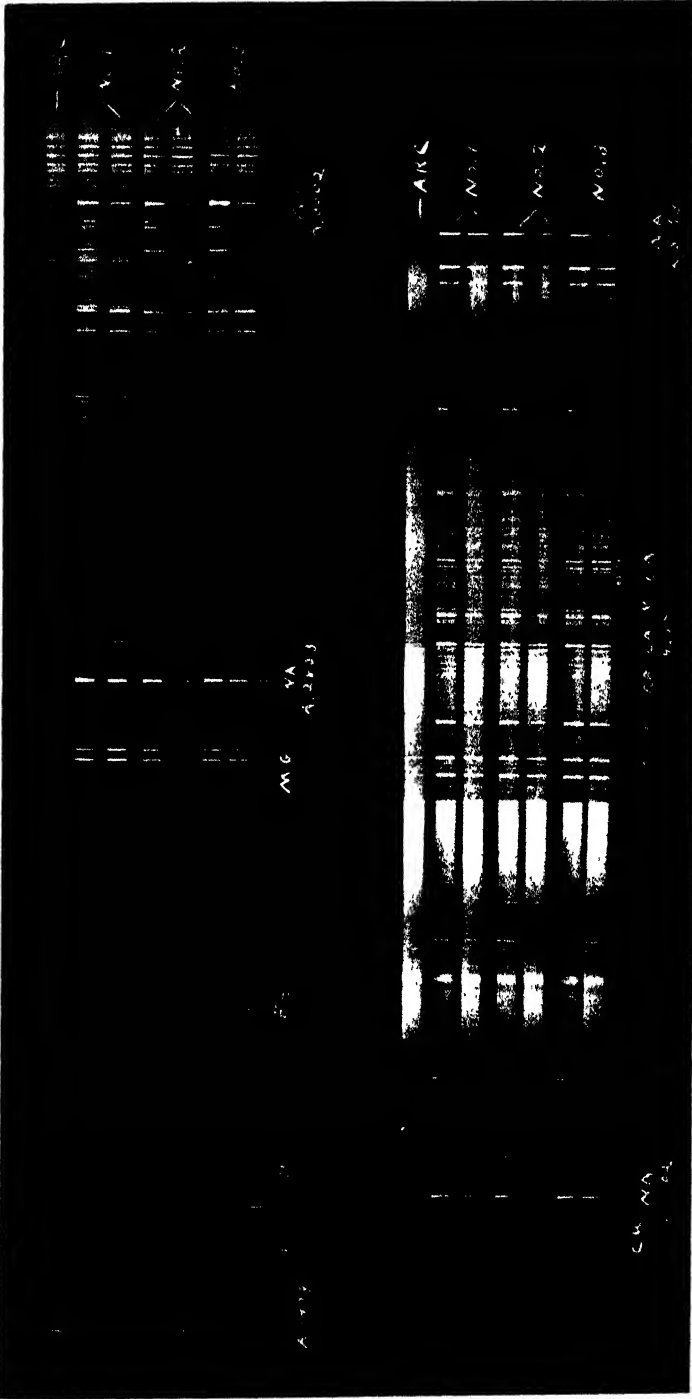


PLATE A.

SPECTROGRAMS OF LIQUID MILK ASH AND DRY MILK ASH.

No. 1. Spectrogram of a single sample of ash from roller process dry milk; No. 2, spectrogram of the ash from a composite sample of roller process dry milk; No. 3, spectrogram of the ash of liquid milk used for making the dry milk No. 1.

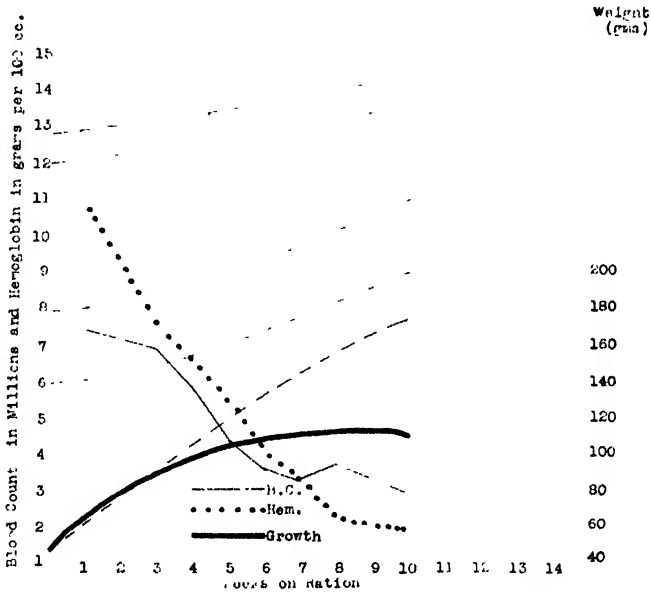


CHART 1.—Group I—Results from Continuous Feeding of Liquid Whole Milk. (Copper 0.64 p.p.m., Iron 1.35 p.p.m.)

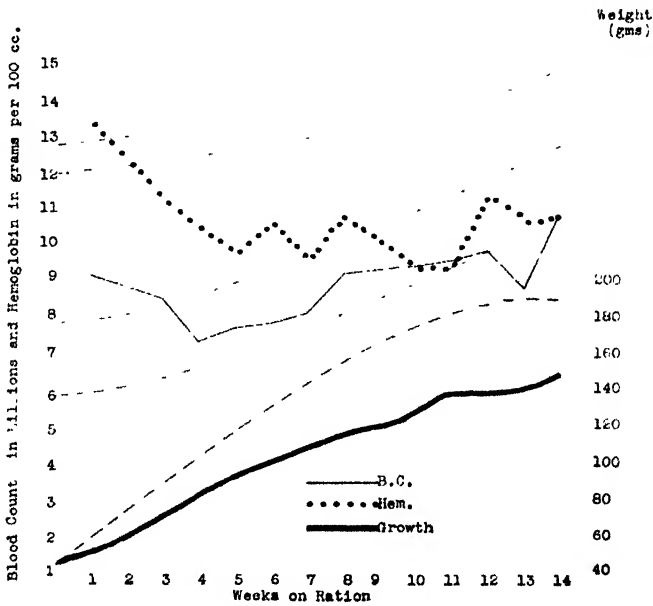


CHART 2.—Group II—Results from Continuous Feeding of Reconstituted Dry Milk Taken from Desiccating Cylinders Polished Daily. (Copper 0.595 p.p.m.; Iron 2.52 p.p.m.)

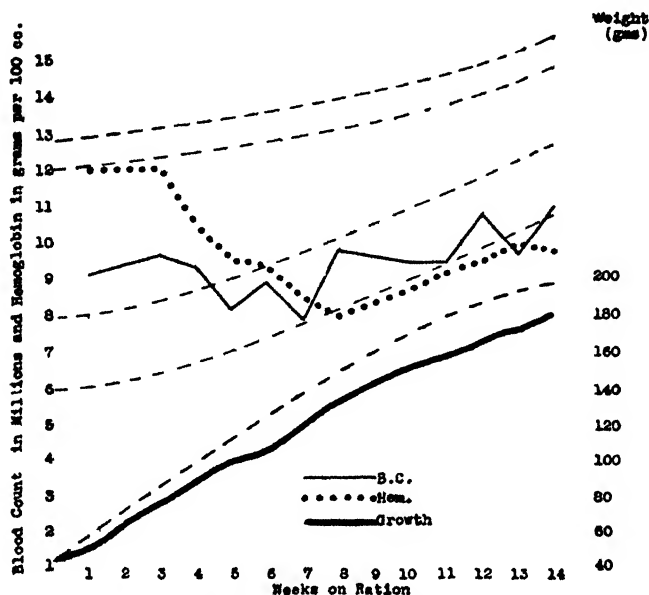


CHART 3.—Group III—Results from Continuous feeding of Reconstituted Dry Milk Taken from Desiccating Cylinders not Polished. (Copper 0.55 p.p.m.; Iron 2.00 p.p.m.)

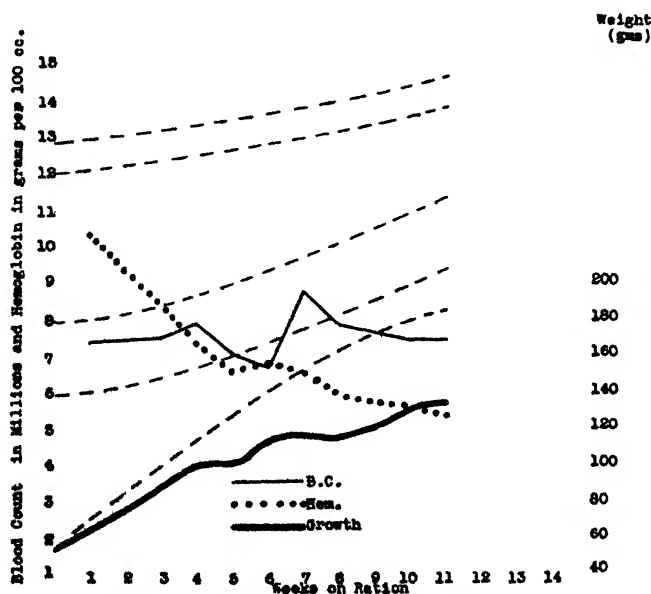


CHART 4.—Group IV—Results from Continuous Feeding of Liquid Milk Supplemented with Liquid Milk Ash—Copper in Liquid Milk Without Supplement 0.665 p.p.m.; Iron 1.18 p.p.m. (The ash from 75 cc. of the liquid milk was added to each 100 cc. of the liquid milk diet.)



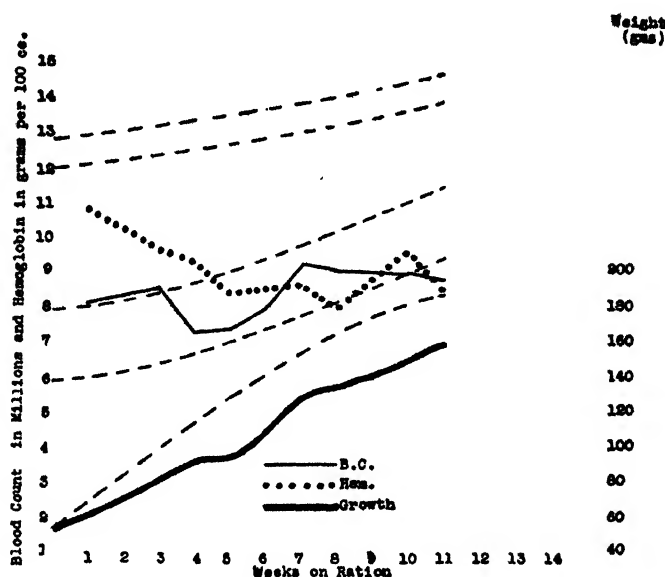


CHART 5.—Group V—Results from Continuous Feeding of Liquid Milk Supplemented with the Ash of Dry Milk Taken from Desiccating Cylinders Polished Daily—Copper in Liquid Milk Without Supplement 0.665 p.p.m.; Iron 1.18 p.p.m. (The ash from 75cc. of reconstituted dry milk—Copper 0.595 p.p.m.; Iron 2.52 p.p.m.—was added to each 100 cc. of the liquid milk diet.)

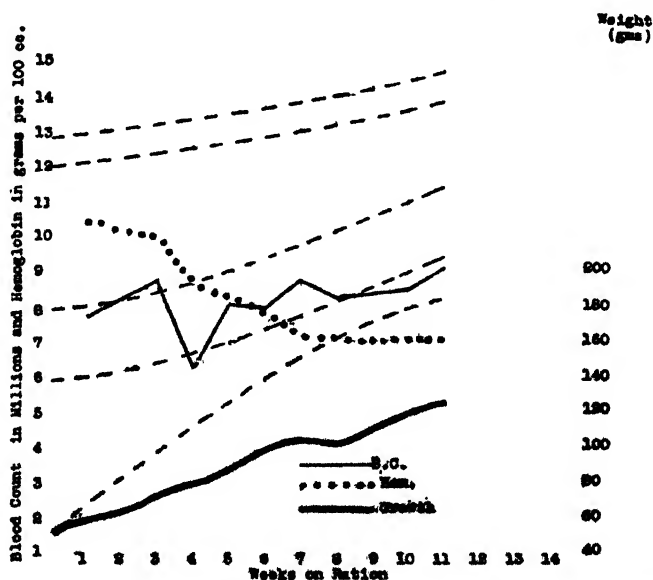


CHART 6.—Group VI—Results from Continuous Feeding of Liquid Milk Supplemented with the Ash of Dry Milk Taken from Desiccating Cylinders not Polished. Copper in Liquid Milk Without Supplement 0.665 p.p.m.; Iron 1.18 p.p.m. (The ash from 75 cc. of reconstituted dry milk—Copper 0.55 p.p.m.; Iron 2.00 p.p.m.—was added to 100 cc. of the liquid milk diet.)

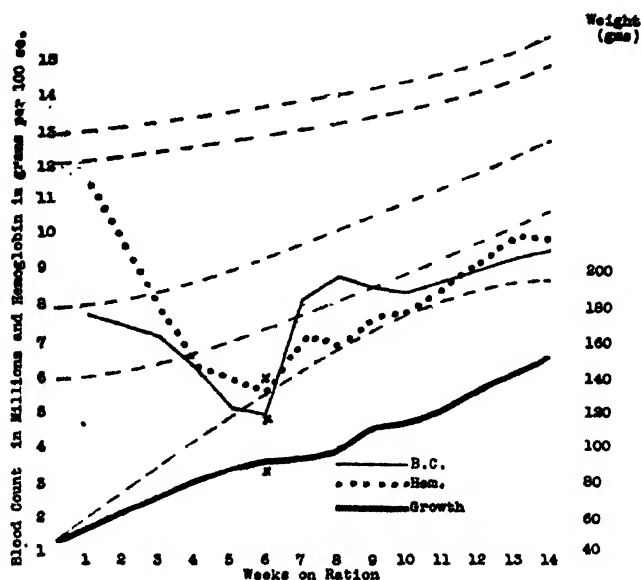


CHART 7.—Group VII—Results Showing Development of Anemia by Feeding Fluid Milk and Subsequent Improvement After Changing to Reconstituted Dry Milk Taken from Desiccating Cylinders Polished Daily. (Copper in liquid milk 0.665 p.p.m.; Iron 1.18 p.p.m.—Copper in reconstituted dry milk 0.595 p.p.m.; Iron 2.52 p.p.m.)

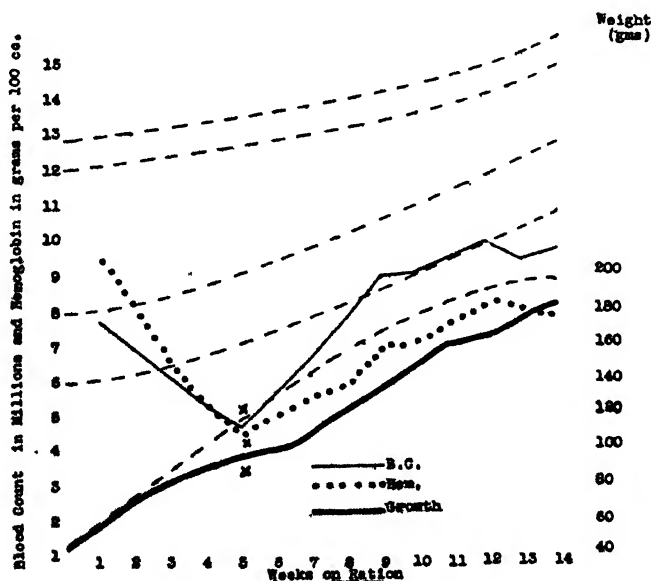


CHART 8.—Group VIII—Results Showing Development of Anemia by Feeding Fluid Milk and Subsequent Improvement after Changing to Reconstituted Dry Milk Taken from Desiccating Cylinders not Polished. (Copper in liquid milk 0.665 p.p.m.; Iron 1.18 p.p.m.—Copper in reconstituted dry milk 0.55 p.p.m.; Iron 2.00 p.p.m.)

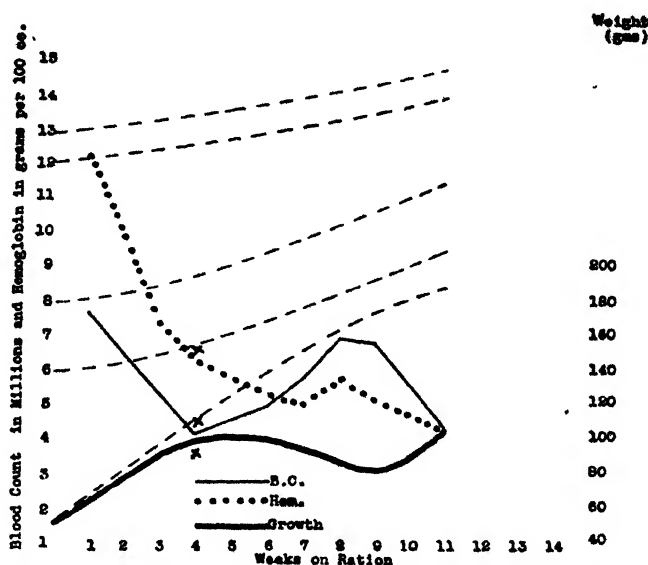


CHART 9.—Group IX—Results Showing Development of Anemia by Feeding Liquid Milk and Negligible Subsequent Effect After Supplementing with Liquid Milk Ash. Copper in Liquid Milk Without Supplement 0.665 p.p.m.; Iron 1.18 p.p.m. (The ash from 75 cc. of liquid milk—Copper 0.665 p.p.m.; Iron 1.18 p.p.m.—was added to each 100 cc. of liquid milk diet.)

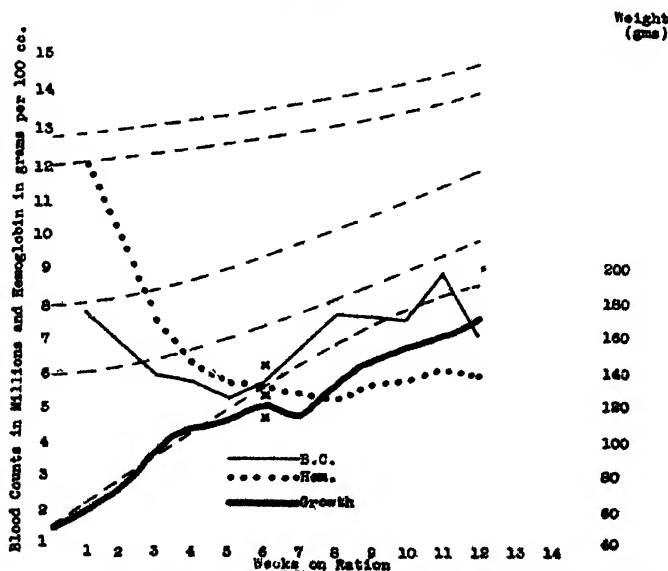


CHART 10.—Group X—Results Showing Development of Anemia by Feeding Liquid Milk and Negligible Subsequent Effect after Supplementing with Dry Milk Ash Obtained from Powder Taken from Deaerating Cylinders Polished Daily. Copper in Liquid Milk Without Supplement 0.665 p.p.m.; Iron 1.18 p.p.m. (The ash from 75 cc. of reconstituted dry milk—Copper 0.595 p.p.m.; Iron 2.52 p.p.m.—was added to 100 cc. of the liquid milk diet.)

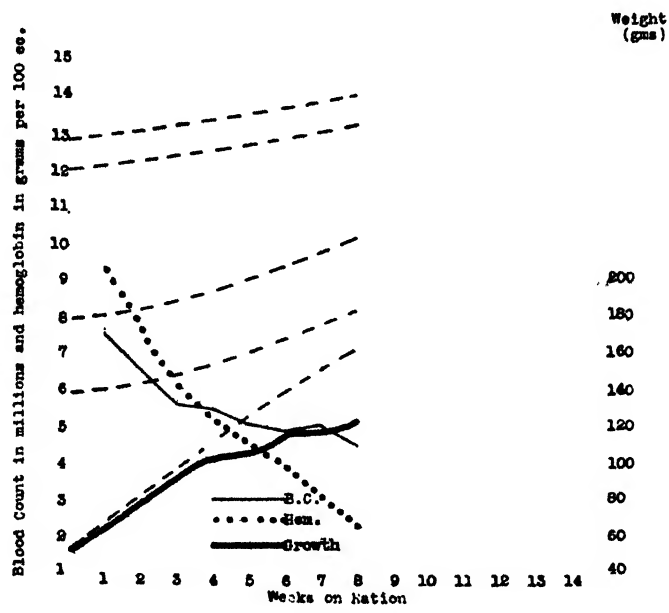


CHART 11.—Group XI—Results from Continuous Feeding of Liquid Milk Boiled for two Minutes. (Copper 0.575 p.p.m.; Iron 1.31 p.p.m.)

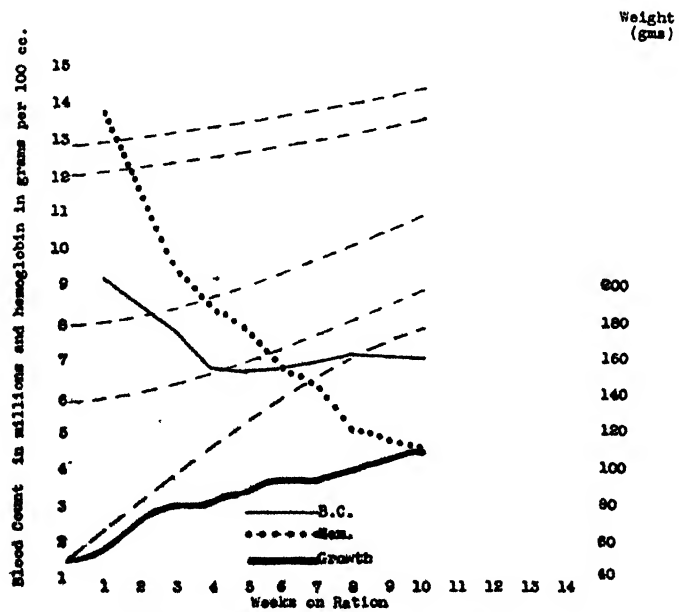


CHART 12.—Group XII—Results from Continuous Feeding of Reconstituted Spray Process Dry Milk. (Copper 0.64 p.p.m.; Iron 1.23 p.p.m.)

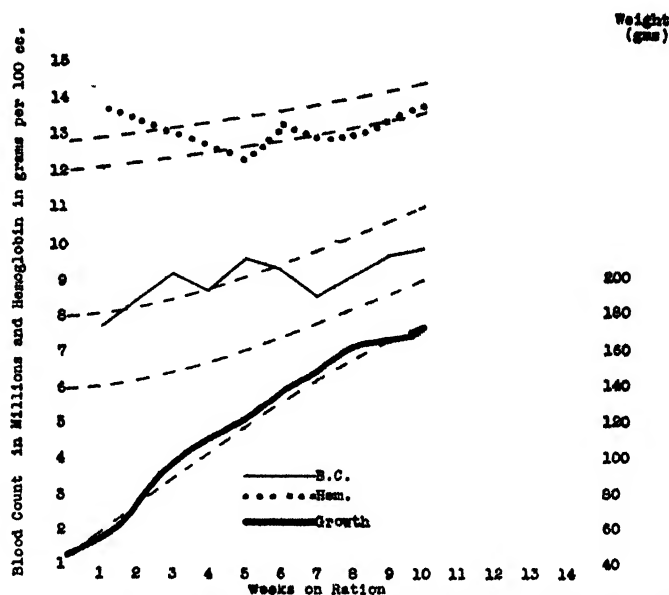


CHART 13.—Group XIII—Results from Continuous feeding of Spray Process Dry Milk Which was Reconstituted to the Liquid Basis and Again Dried by the Cylinder Process Before Feeding. (Copper 0.64 p.p.m.; Iron 4.37 p.p.m.)

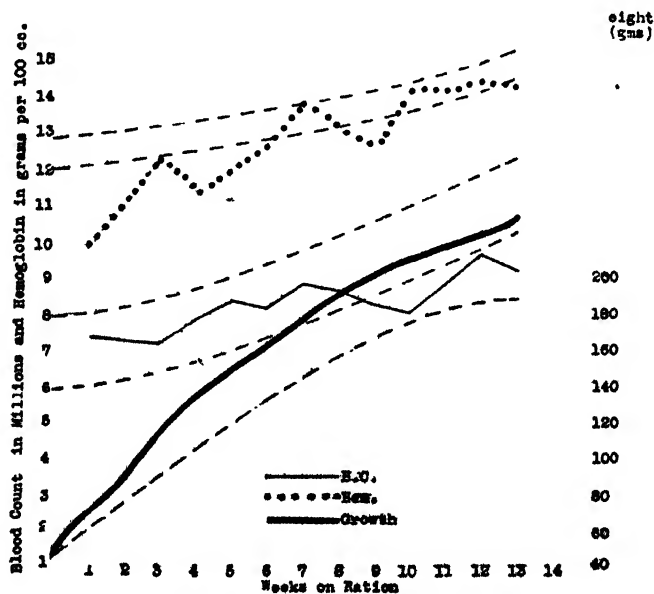


CHART 14.—Group XIV—Results from Animals Receiving the Regular Stock Ration. (Grain mixture, Copper 10 p.p.m.; Iron 77.7 p.p.m.; whole milk, Copper 0.485 p.p.m.; Iron 1.19 p.p.m.)

## REFERENCES

1. Bertrand, G., *Bull. soc. hyg. alimentaire*, VIII, 49, 1920. (*Chem. Abstr.* XIV, 3450, 1920.)
2. Supplee, G. C., and Bellis B., *Jour. Dairy Sci.*, V, 455, 1922.
3. Hess, A. F., Supplee, G. C., and Bellis B., *Jour. Biol. Chem.*, LVII, 725, 1923.
4. Rice, F. E., and Miscall, J., *Jour. Dairy Sci.*, VI, 261, 1923.
5. Quam, G. N., and Hellwig, A., *Jour. Biol. Chem.*, LXXVIII, 681, 1928.
6. McHargue, J. S., Healy, D. J., and Hill, E. S., *Jour. Biol. Chem.*, LXXVIII, 637, 1928.
7. Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A. *Jour. Biol. Chem.*, LXVIII, 797, 1928.
8. Waddell, J., Steenbock, H., Elvehjem, C. A., and Hart, E. B., *Jour. Biol. Chem.*, LXXVII, 769, 1928.
9. Waddell, J., Steenbock, H., and Hart, E. B., *Jour. Biol. Chem.*, LXXXIII, 243, 1929.
10. Waddell, J., Steenbock, H., Elvehjem, C. A., and Hart E. B., *Jour. Biol. Chem.*, LXXXIII, 251, 1929.
11. Krauss, W. E., *Jour. Dairy Sci.*, XII, 74, 1929.
12. Titus, R. W., and Hughes, J. S., *Jour. Dairy Sci.*, XII, 90, 1929.
13. Elden, C. A., Sperry, W. M., Robsheit-Robbins, F. S., and Whipple, G. H., *Jour. Biol. Chem.*, LXXIX, 577, 1928.
14. Drabkin, D. L., and Waggoner, C. S., *Science*, LXIX, 480, 1929.
15. *LeLait Desseche*, 2nd. edition, by Chr. Porcher, Published Lyon France, 1926. (English translation now in press.)
16. Supplee, G. C., *Proceedings World's Dairy Congress*, II, 1248, 1923.
17. Elvehjem, C. A., Steenbock, H., and Hart, E. B., *Jour. Biol. Chem.*, LXXXIII, 27, 1929.
18. Bodansky, M., *Jour. Biol. Chem.*, XLVIII, 361, 1921.
19. Miller, R. C., Forbes, E. B., and Smythe, C. V., *This Jour.*, I, 217, 1929.





## THE EFFECT OF LOW CALCIUM, HIGH MAGNESIUM DIETS ON GROWTH AND METABOLISM OF CALVES

BY C. F. HUFFMAN, C. S. ROBINSON, O. B. WINTER,  
AND R. E. LARSON

*(From the Department of Dairy Husbandry and Chemical Laboratory  
of the Michigan Agricultural Experiment Station, East Lansing, Mich.)\**

Received for Publication—October 10, 1929

SINCE its publication by Oscar Loew (1) the theory of calcium-magnesium antagonism has been widely accepted. It has been confirmed by Meltzer and Auer (2) and the results of many other investigators have supported it. Should it be true that the utilization of calcium is seriously impaired by a disturbance in the calcium-magnesium ratio, the inclusion in a diet of too great a proportion of magnesium-rich ingredients; *e.g.*, grain concentrates or high-magnesium mineral supplements, might produce serious results.

The evidence available does, indeed, indicate that the injection of magnesium salts (3) or their feeding (4) actually causes some decrease in calcium retention but its importance in the animal economy has been questioned, particularly if the ration supplies an abundance of calcium (5) or of phosphorus (8, 9, 10).

The purpose of the present investigation was to study the effect of magnesium when fed in connection with low calcium diets particularly after these diets had produced certain pathological conditions under which the deleterious action of the magnesium should be more marked than in normal animals.

### THE EFFECT OF FEEDING MAGNESIUM PHOSPHATE

Calves fed from birth on whole milk, or whole milk and grain, usually die in convulsions. McCandlish (6) found that calves died earlier when whole milk was supplemented with grain. He suggested that this was due to the depletion of the body stores of calcium. The fact that grains contain more magnesium than calcium causes calcium to be drawn from the body, especially the bones, to take care of the excess of magnesium. He also stated that it would appear that part of the beneficial effect of alfalfa added to a ration of milk and grain is that it increases the calcium as compared with the magnesium in the ration and thus conserves the calcium stores of the body.

\* Published with the permission of the Director of the Experiment Station as Journal Article No. 15 (N.S.).



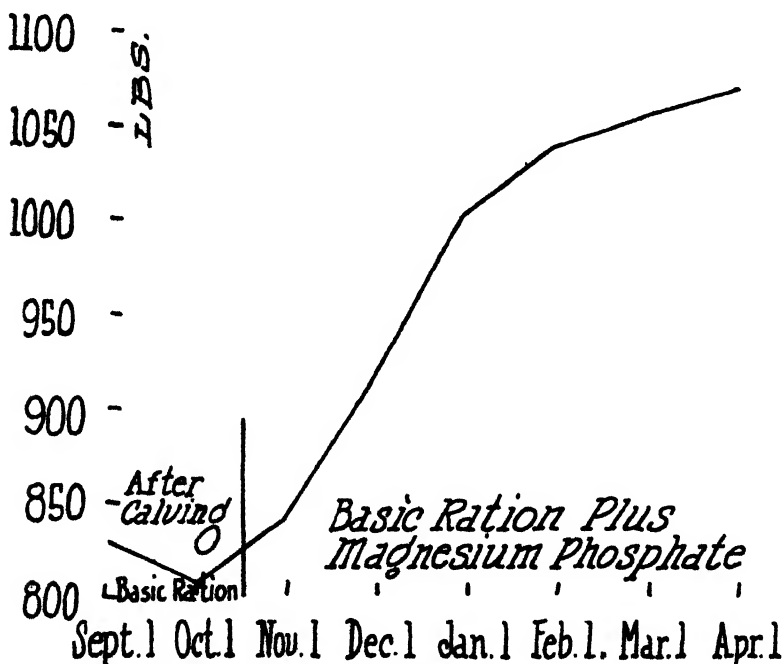
A grade Holstein calf C8 was raised on milk alone until it developed the signs of irritability which usually precede convulsions. Fifty grams of magnesium phosphate were added to the ration in an attempt to intensify the condition. However, the addition of magnesium phosphate instead of increasing irritability temporarily relieved it. The magnesium phosphate was increased whenever the animal manifested evidence of irritability until at 242 days of age 175 grams were being fed daily as shown in Table I. This animal died at 262 days of age but not in a convulsion.

TABLE I.  
SHOWING GROWTH IN WEIGHT AND FOOD CONSUMPTION OF CALF C8.

10 Day Periods	Age Days	Weight lbs.	Percent Normal	Whole Milk lbs. in 10 da.	Magnesium Phosphate gms. in 10 da.
1	12	93	90.8	76.0	—
2	22	97	86.1	92.0	—
3	32	107	86.7	102.0	—
4	42	117	87.1	120.0	—
5	52	128	86.8	120.0	—
6	62	138	86.3	120.0	—
7	72	146	83.8	134.0	—
8	82	151	80.1	140.0	—
9	92	158	77.7	126.0	—
10	102	168	85.2	150.0	—
11	112	178	75.4	176.0	50.
12	122	189	74.8	200.0	500.
13	132	203	75.3	200.0	500.
14	142	207	71.9	200.0	1000.
15	152	228	74.7	200.0	1000.
16	162	237	73.9	200.0	1000.
17	172	242	71.9	200.0	1000.
18	182	250	71.1	200.0	1000.
19	192	265	72.6	213.0	1000.
20	202	285	75.4	220.0	1000.
21	212	293	74.8	220.0	1025.
22	222	296	73.4	234.0	1300.
23	232	317	76.3	240.0	1300.
24	242	324	75.7	240.0	1750.
25	252	334	75.7	240.0	1750.
26	262	340	74.7	169.0	1225.

The following ration was fed heifer No. 205 at one year of age; 400 pounds yellow corn; 50 pounds corn gluten meal, 50 pounds cottonseed meal, 5 pounds salt, and 25 pounds wheat straw. She gave birth, after 234 days of gestation, to a blind, partially paralyzed calf which lived only two days. She also had a retained placenta.

Fifty days following parturition magnesium phosphate (which contained 0.0015 per cent calcium) was added to her basal ration at the rate of 5 per cent of the grain mixture. She was in an emaciated condition at this time. The ingested magnesium phosphate produced a slight purgative effect. There was an immediate improvement in body weight as shown in Graph 1. Her appearance also changed markedly. Unfortunately she reacted to



GRAPH 1.—Showing the gain in body weight of Cow 205 after the addition of magnesium phosphate to a ration low in calcium.

the agglutination test for *B. abortus* infection, which necessitated her removal from the experiment before calving a second time. Although her ration was very low in calcium, the addition of magnesium phosphate not only failed to aggravate her symptoms, but apparently brought about an improvement in the health and well-being of the animal.

Five grade Holstein heifers (C35, C37, C38, C39, and C46) were placed on an experiment at four months of age, up to which time they had received a recognized adequate ration. In the experiment C35, C38, and C46 were fed a basal ration consisting of wheat straw, corn silage, and the following grain ration; 3 parts yellow corn, 1 part oats, 1 part cottonseed meal, and 1 per cent salt. The other two animals, C37 and C39, received the basal ration plus 3 per cent of the grain mixture as magnesium phos-

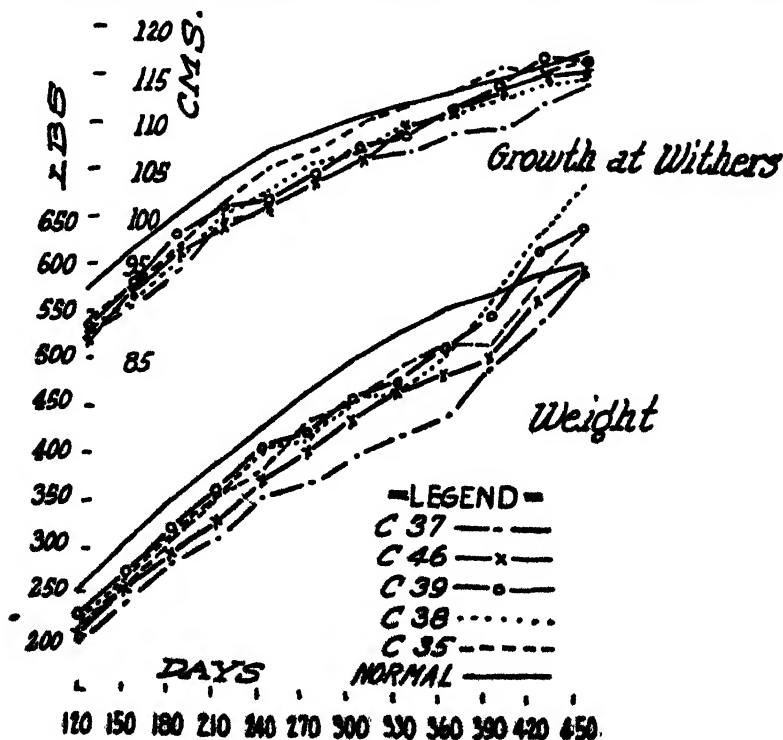
phate, a ration low in calcium and high in magnesium. The calcium, magnesium and phosphorus analyses of the feed and water fed these animals are shown in Table II.

TABLE II  
SHOWING COMPOSITION OF FEEDS

	% Ca	% P	% Mg
Grain mixture (basic)	0.086	0.271	0.48
Grain mixture plus magnesium phosphate	0.086	0.664	0.951
Corn silage	0.100	0.070	0.061
Wheat straw	0.293	0.181	0.070
Water	0.0085	—	0.0029

Wheat straw was fed *ad libitum* the first 280 days of the experiment. After that it was cut into short pieces and weighed and the animals were fed all they would clean up.

The animals were weighed at intervals of 10 days. At the end of every



GRAPH 2.—Showing growth in weight and height at withers of heifers C35, C38, and C46, fed basic ration low in calcium; and heifers C37 and C39 on basic ration supplemented with magnesium phosphate.

30 day period, they were weighed 3 days in succession. Weighing was done in all cases in the morning before feeding and watering. The following measurements were taken at 30 days intervals; height at withers, height at rump, circumference of chest (just behind the shoulders), greatest circumference of barrel, depth of chest, width of chest, width of barrel, width of hooks, and width of thurls. The growth of these animals from 4 months to 15 months of age, as manifested by body weight and height at withers, is shown in Graph 2.

*Calcium, Phosphorus, and Magnesium Metabolism.* Each of the above animals was placed on metabolism experiment for a period of 7 days in order to determine the balance of calcium, magnesium, and phosphorus. This was done during November and December. They were placed in the metabolism stalls two days before the actual starting of the experiment in order to get them accustomed to the change. The animals were exercised in the open 20 minutes each day during the metabolism period.

The feces and urine, collected separately in suitable containers by attendants, were weighed or measured at the end of each 24 hours and representative samples of each taken for analysis. The methods of analysis were the same as in previous work (7). The results which are summarized in Table III showed that magnesium, as such, did not exert the harmful effect sometimes attributed to it. However, the experiments were not so conclusive as they might have been had some salt other than the phosphate been used, since Hart and Steenbock (8) and Haag and Palmer (9) have shown that phosphorus counteracts the ill effects of magnesium. Medes (10) has also shown that the ratios of Ca, P and Mg may be varied through rather wide ranges without producing visible abnormalities in rats unless the absolute amount of phosphorus is low. C35 and C37 were kept on the experiment for three years. During lactation, a seven-day metabolism period indicated that both animals were utilizing calcium efficiently even though C37 was receiving a high magnesium ration.

#### EFFECT OF FEEDING MAGNESIUM CARBONATE

A Holstein bull calf, C44, was fed a ration low in calcium but adequate in phosphorus which consisted of 2 parts ground yellow corn, 2 parts ground oats, 1 part corn distiller's grains, 4 parts corn gluten meal, 1 per cent salt and wheat straw *ad lib*. This animal became blind at about 11 months of age. At about 13 months of age the joints became enlarged and stiffness developed. This condition became very severe in June, although the animal was turned into an open lot for about 7 hours per day.

After he became so stiff that assistance was necessary to get him up from a lying position, 135 grams of commercial magnesium carbonate amounting to 5 per cent of the grain ration was added daily in an attempt to aggravate the condition. This addition was made July 7 when the animal was 467 days of age. The ingested magnesium carbonate had a purgative effect and the stools which had been firm and black became soft. An immediate improvement in health was also observed. Coincident with this, the appetite increased so that the grain fed daily was gradually increased to 9 pounds per day.

The body weight, which had remained practically constant for about four months before the addition of magnesium carbonate, gradually in-

TABLE III

SHOWING AVERAGE DAILY CALCIUM, PHOSPHORUS AND MAGNESIUM BALANCES OF ANIMALS ON A BASIC RATION LOW IN CALCIUM AND BASIC RATION SUPPLEMENTED WITH MAGNESIUM PHOSPHATE

		Animal	Feces	Urine	Milk	Total Outgo	Intake	Plus Balance	Percent Utilized	Ca/P in food
Calcium	Period 1	C 35	5.79	0.67	—	6.45	13.47	7.02	52.1	0.71
		C 35*	8.72	2.19	21.9	32.80	41.97	9.16	74.0	0.95
		C 38	6.57	0.52	—	7.09	13.55	6.46	47.7	0.71
		C 46	3.65	0.16	—	3.81	9.06	5.26	58.0	0.55
	Period 2	C 39	5.94	0.15	—	6.09	13.63	7.55	55.4	0.40
		C 37	5.38	0.75	—	6.14	13.09	6.96	53.1	0.39
		C 37*	11.70	2.84	13.56	28.10	34.10	6.00	57.4	0.50
Phosphorus	Period 1	C 35	12.53	2.73	—	15.25	19.05	3.80	19.9	
		C 35*	18.80	4.66	15.38	38.84	44.26	5.42	47.0	
		C 38	10.19	4.31	—	14.50	19.03	4.53	23.8	
		C 46	8.03	4.74	—	12.77	16.55	3.78	22.8	
	Period 2	C 39	19.06	9.03	—	28.09	34.04	5.95	17.5	
		C 37	17.20	10.98	—	28.18	33.67	5.49	16.3	
		C 37*	39.30	4.53	10.45	54.28	68.58	14.30	36.1	
Magnesium	Period 1	C 35	14.31	1.83	—	16.13	16.70	0.57	3.4	
		C 35*	16.18	11.72	1.50	29.40	31.28	1.88	10.7	
		C 38	13.21	2.17	—	15.38	16.70	1.32	7.9	
		C 46	10.04	2.20	—	12.24	13.02	0.78	6.0	
	Period 2	C 39	23.62	3.11	—	26.73	29.21	2.48	8.5	
		C 37	22.20	2.71	—	24.90	28.60	3.70	12.9	
		C 37*	35.24	8.11	0.62	43.97	52.62	8.65	16.4	

Period No. 1, basic ration. Period No. 2, basic ration plus magnesium phosphate.

\* During first lactation the basic ration consisted of 2 parts yellow corn, 2 parts linseed oil meal, 1 part oats, wheat, straw and salt.



FIG. 1.— Showing animal C44 after having received commercial magnesium carbonate as a supplement to a basic ration low in calcium for one year.



FIG. 2.— Showing animal C44 about six months after commercial magnesium carbonate had been withdrawn from the ration.

creased. The magnesium carbonate was reduced to 3 per cent of the grain mixture on September 5, at which level it was continued till July 14, 1927. At this time the animal appeared normal in health and appearance, as shown in Fig. 1. The stiffness had completely disappeared.

After magnesium carbonate had been withdrawn he appeared normal for about two months when signs of stiffness again appeared. This condition became severe in December. In January 1928, he was able to get up only with considerable difficulty. The appetite was also affected adversely and his general condition was poor (Fig. 2). He was slaughtered February 14. The rib bones were soft and easily broken near the sternal ends.

The weight, volume, specific gravity, and breaking strength of some of the long bones are shown in Table IV. The volume of the bones was deter-

TABLE IV  
SHOWING WEIGHT, SPECIFIC GRAVITY, AND BREAKING STRENGTH OF SOME OF THE LONG BONES  
OF CALF C44

Bones	Weight gms.	Volume cc	Specific Gravity	Breaking Strength lbs.
Right femur	2200	1900	1.16	3,125
Left femur	2350	1985	1.18	2,000
Right humerus	1775	1530	1.16	2,935
Left humerus	1705	1445	1.18	2,660
Right large metatarsis	485	373	1.30	3,420
Left large metatarsis	477	353	1.35	3,705
Right large metacarpis	435	330	1.32	2,585
Left large metacarpis	433	325	1.33	2,390

mined by water displacement method. The breaking strength was determined by breaking a 6 inch section of the shaft. The specific gravity of these bones was much less than values we have found for bones of similar size from normal animals.

Calcium and phosphorus balances were determined for a seven-day period when magnesium carbonate was being fed and again without this mineral supplement. These results are shown in Table V.

From Table V it is apparent that the magnesium furnished by the magnesium carbonate was not antagonistic to calcium and phosphorus utilization since 10.38 grams of calcium and 7.80 grams of phosphorus were stored during the magnesium carbonate period, while during the period without magnesium carbonate there was a loss of 2.55 grams of calcium and 2.15 grams of phosphorus per day.

*Calcium, Magnesium and Phosphorus Balances.* In order to determine

TABLE V

SHOWING AVERAGE DAILY CALCIUM AND PHOSPHORUS BALANCE OF CALF C44 ON BASIC RATION  
LOW IN CALCIUM AND BASIC RATION SUPPLEMENTED WITH MAGNESIUM CARBONATE

	Period	Feces	Urine	Milk	Total Outgo	Intake	Balance	Percent Utilized	Ca/P in food
Calcium	1	3.39	0.32	—	13.71	11.16	-2.55		0.64
	2	3.67	0.37	—	4.05	14.45	10.38	71.93	0.83
Phosphorus	1	17.69	1.84	—	19.53	17.38	-2.15		
	2	4.96	4.62	—	9.58	17.38	7.80	44.87	

Period No. 1, basic ration. Period No. 2, basic ration plus  $MgCO_3$ .

the effect on the calcium and phosphorus metabolism of feeding magnesium carbonate, three mature Holstein cows which were in the last stage of lactation were placed on a basic ration of timothy hay, a grain mixture containing 3 parts yellow corn, 1 part ground oats, 3 parts linseed oil meal, and 1 per cent salt. After a preliminary feeding period of 1 week, a 7 days' metabolism period was run, after which 150 grams of magnesium carbonate were fed as a mineral supplement per day per cow for 7 days (period 2) and the calcium, phosphorus, and magnesium balances were determined for both periods. However, in determining the average daily balances in period 2, only the last 5 days were used.

The cows were fed grain and hay and watered twice a day. The timothy hay was of good quality from the standpoint of color and purity. The college water supply, which contains 0.0085 per cent calcium, 0.0029 per cent magnesium and only a trace of phosphorus, was used. Commerical magnesium carbonate, which contained 1.37 per cent calcium and 26.05 per cent magnesium, was used. The animals had voracious appetites throughout the experiment.

The collection of excreta, and the sampling and analysis of excreta and feed were carried out as before. A composite sample of milk was taken for each 7 day period. The results are shown in Table VI.

## DISCUSSION

The experiments described in this paper afforded no evidence of any bad effects from including 3 to 5 per cent magnesium salt in the ration. On the contrary, in every case the general health of the animals and, when



TABLE VI

SHOWING AVERAGE DAILY CALCIUM, PHOSPHORUS, AND MAGNESIUM BALANCES OF DAIRY COWS WITH AND WITHOUT MAGNESIUM CARBONATE AS A MINERAL SUPPLEMENT

		Animal	Feces	Urine	Milk	Total Outgo	Intake	Balance	Per cent Utilized	Ca/P in food
Calcium	Period 1	155	25.78	1.26	3.75	30.79	33.06	2.28	18.2	0.95
		143	26.12	1.63	4.93	32.68	33.66	0.98	2.9	0.97
		188	22.82	1.15	3.98	27.95	33.02	5.07	27.4	0.95
	Period 2	155	23.12	0.72	2.87	26.71	35.31	8.59	32.5	1.02
		143	26.49	1.51	4.88	32.87	35.91	3.04	22.0	1.04
		188	24.63	1.06	2.90	28.59	34.94	6.35	26.5	1.01
Phosphorus	Period 1	155	29.76	0.19	3.13	33.08	34.65	1.57	13.6	
		143	30.42	0.27	3.94	34.63	34.65	0.02	0.05	
		188	27.98	0.21	3.02	31.21	34.65	3.44	1.87	
	Period 2	155	24.91	0.22	2.40	27.53	34.65	7.11	27.4	
		143	30.07	0.21	3.86	34.14	34.65	0.51	12.6	
		188	26.70	0.34	2.18	29.22	34.65	5.43	21.9	
Magnesium	Period 1	155	15.92	4.00	0.58	20.50	24.31	3.80	18.0	
		143	12.92	5.56	0.78	19.27	24.51	5.24	24.5	
		188	15.99	3.88	0.48	20.35	24.29	3.94	18.2	
	Period 2	155	39.67	5.84	0.41	45.92	63.45	17.53	27.8	
		143	41.02	10.05	0.72	51.79	63.66	11.87	19.8	
		188	39.41	8.64	0.30	48.35	63.33	14.98	24.1	

Period No. 1, basic ration. Period No. 2, basic ration plus  $MgCO_3$ .

it was determined, the calcium and phosphorus retention, were unaffected or were improved.

In the case of the calf C8, which showed characteristic nervous disturbances ascribed by McCandlish to too much magnesium, the addition of magnesium phosphate gave relief instead of aggravating the symptoms. We have shown that these disturbances are accompanied by a hypocalcemia (11) which gives the present experiment a resemblance to those of Wenner (12) who reported that the continuous oral administration of magnesium lactate was an effective agency in preventing tetany in parathyroidectomized dogs. Luckhardt and his coworkers likewise prevented the onset of convulsions in thyroparathyroidectomized dogs by the administration of magnesium chloride. This experiment is complicated by the fact that the chloride probably exerted a beneficial effect through its acidogenic function (13) but the fact remains that the magnesium effect

TABLE VII  
EFFECT OF MG ON BLOOD PICTURE OF A CALF WITH OSTEOMALACIA

Period	No. of Sample	Ca	P	Remarks
1925				
4/10-6/10	9	11.6	5.58	
6/10-8/10	7	10.8	6.87	
8/10-10/10	9	10.1	7.17	
10/10-12/10	21	10.4	6.28	
1926				
12/10-2/10	10	8.2	5.62	
2/10-4/10	9	8.7	3.29	Stiff
4/10-6/10	12	8.4	3.03	"
6/10-8/10	6	10.7	7.83	MgCO <sub>3</sub> , 5% of ration
8/10-10/10	6	10.6	6.68	" 3% "
10/10-12/10	9	10.3	7.68	" " "
1927				
12/10-2/10	7	10.6	6.26	" " "
2/10-4/10	9	9.7	6.87	" " "
4/10-6/10	9	9.7	7.55	" " "
6/10-7/13	5	9.2	7.34	MgCO <sub>3</sub> discontinued
7/20-10/10	11	8.0	6.24	
10/10-12/10	9	9.3	3.62	Stiff
1928				
12/10-2/10	9	8.9	3.13	Stiff

was not potent enough to change the results which might have been expected from those produced by the analogous calcium salt.

The addition of magnesium phosphate to the ration of No. 205, which was low in calcium and fairly high in phosphorus, resulted in a marked improvement in body weight and health. Although she showed no rachitic symptoms, her physical condition and reproductive failure are evidence of the inadequacy of the ration. Her response to magnesium feeding was comparable to that of C44 which did show rachitic symptoms.

The case of C44 is so unique in many respects that it deserves special attention. In the first place, an animal on a low calcium, high phosphorus, diet should develop a condition characterized by low blood calcium rather than low blood phosphorus. The picture actually produced is identical with the rachitic condition<sup>1</sup> described by Karelitz and Shohl (14) for rats and Shohl and Bennet (15) for dogs on a *high calcium, low phosphorus* diet.

<sup>1</sup> Whether or not our subject developed a true case of rickets we are unable to state because of insufficient histological data, but the symptoms and blood picture were typical and the gross appearance of the bones showed skeletal abnormalities resembling rickets.

The addition of 3 to 5 per cent of  $MgCO_3$  to the diet produced a clear-cut improvement in the condition of the animal coincident with the alteration of the blood picture. This is directly contradictory to the findings of Park and his associates who were able to produce typical low-phosphorus rickets "by the addition to the diet of magnesium carbonate in quantities varying between 1 and 4 per cent" (16). They are also at variance with the recently reported results of Elmslie and Steenbock (17) who, though finding no positive deleterious action with magnesium carbonate, found no curative action.

Two factors must be considered in connection with this animal, the difference in calcium intake and the laxative action of the magnesium carbonate.

During the period when the magnesium was being fed, the calcium intake was 14.45 grams per day of which 10.38 grams were stored. When magnesium was discontinued, the calcium intake was reduced to 11.16 grams. Simultaneously the storage dropped to -2.55 grams and the animal became stiff. The discrepancy in calcium storage in the two cases indicates that it was not the slight reduction in calcium intake that was responsible for the disturbance produced by the withdrawal of the magnesium. This position is supported by the fact that the phosphorus storage also fell from 7.80 to -2.15 grams per day. The importance of the second factor cannot be estimated but it should have been as active in the experiments of the other investigators cited above as in ours. This experiment is being repeated with several animals.

While the metabolism results with the above animals showed more pronounced beneficial results from the feeding of magnesium than did the others, none of the latter could be interpreted as indicating a harmful action by this material as no increase in the loss of calcium or phosphorus accompanied its inclusion in the ration.

With the other animals the influence of the magnesium compounds on the paths of excretion was not marked, but in the case of C44 magnesium carbonate reduced the loss of calcium and phosphorus in the feces and, to a smaller extent, increased their excretion in the urine.

The magnesium from the carbonate was mostly eliminated through the bowel though there was a slight increase in urinary magnesium. Either carbonate or phosphate increases the storage of magnesium though the former is apparently more effective in this respect.

#### BIBLIOGRAPHY

1. Loew, O., *Landw. Versuch-Sta.* XL, 467, 1892.
2. Meltzer, S. J., and Auer, J., *Amer. Jour. Physiol.*, XXI, 400, 1908.

3. Mendel, L. B., and Benedict, S. R., *Amer. Jour. Physiol.*, XXV, 23, 1909.
4. Underhill, F. P., Honeij, J. A., and Bogert, L. J., *Jour. Exp. Med.*, XXXII, 65, 1920.
5. Bogert, L. J., and McKittrick, E. J., *Jour. Biol. Chem.*, LIV, 363, 1922.
6. McCandlish, A. C., *Jour. Dairy Science*, VI, 347, 1923.
7. Robinson, C. S., Huffman, C. F., and Mason, M. F., *Jour. Biol. Chem.*, LXXXIV, 257, 1929.
8. Hart, E. B., and Steenbock, H., *Jour. Biol. Chem.*, XIV, 75, 1913.
9. Haag, J. R., and Palmer, L. S., *Jour. Biol. Chem.*, LXXVI, 367, 1928.
10. Medes, Grace., *Jour. Biol. Chem.*, LXVIII, 295, 1927.
11. Huffman, C. F., and Robinson, C. S., *Jour. Biol. Chem.*, LXIX, 101, 1926.
12. Wenner, W. F., *Amer. Jour. Physiol.*, LXXXI, 392, 1927.
13. Luckhardt, A. B., Ward, R. O., and Brannon, L., *Amer. Jour. Physiol.*, LXXVI, 228, 1926.
14. Karelitz, S., and Shohl, A. T., *Jour. Biol. Chem.*, LXXIII, 665, 1927.
15. Shohl, A. T., and Bennett H., *Jour. Biol. Chem.*, LXXIV, 247, 1927.
16. Park, E. A., *Physiol. Rev.* III, 129, 1923., *Amer. Jour. of Physiol.*, XLXVI, 228, 1926.
17. Elmslie, W. P., and Steenbock, H., *Jour. Biol. Chem.*, LXXXII, 611, 1929.





## DIETARY REQUIREMENTS FOR FERTILITY AND LACTATION

### XXII. FURTHER STUDIES OF THE ROLE OF MILK FAT IN FERTILITY AND LACTATION\*

BY BARNETT SURE

With the assistance of Alethea Beach  
(From the Laboratory of Agricultural Chemistry,  
University of Arkansas, Fayetteville)

Received for Publication—October 16, 1929

IN ORDER to secure more evidence of the effect of the increase of small amounts of butter fat in a ration satisfactory for growth, but not entirely adequate for lactation (1), a series of experiments was initiated during October of 1926, which was continued for five generations. This investigation was completed during March of 1929.

The results of the experiments are summarized in Tables I to III. It will be noted that rations 995, 996, and 997 vary essentially only by 2 per cent of butter fat, the differences in whole wheat and skimmed milk pow-

TABLE I  
PERCENTAGE COMPOSITION OF RATIONS

Ration	Whole wheat	Skimmed milk powder	Whole milk powder	Butter fat	NaCl
995*	61.7	32.0	—	5.0	1.3
996*	59.7	32.0	—	7.0	1.3
997*	59.7	30.0	—	9.0	1.3
998*	66.7	—	32.0	—	—
999	Duplicate of Ration 998				

\* At mating the ration of the fifth generation animals was changed so that two per cent wheat oil replaced an equivalent amount of dextrin.

der being negligible from the standpoint of reducing the proteins and vitamin E. Since, however, dry skimmed milk contains 1.74 per cent residual milk fat left after skimming (2), the total milk fat of rations 995, 996 and 997 would be 5.56, 7.56, and 9.52 per cent respectively. The total milk fat of rations 998, and 999, containing whole milk powder is 9.17, or 8.60 per cent butter fat, and 0.57 per cent residual milk fat (3). The purpose of introducing rations 998 and 999 (999 is a duplicate of 998) was to compare the biological value of the fat-soluble vitamins in milk fat carried by whole milk powder with the fat-soluble vitamin content of skimmed milk powder

\* Research paper No. 141, Journal Series, University of Arkansas.

TABLE II  
FERTILITY AND LACTATION RECORD OF ANIMALS ON RATIONS, COMPOSITION OF WHICH IS GIVEN IN TABLE I

Ration	Generation	Reproduction period (days)	Females	Litters	Young born	Young born alive	Young allowed to be reared	Young Weaned		Remarks
								No.	per cent	
995	1st	286	3	20	175	160	110.	45	41	
	2nd	113	4	4	35	35	24	15	62	One female was sterile.
	3rd	158	3	6	57	57	36	36	100	One female was sterile.
	4th	267	3	12	85	82	63	24	36	
	5th	143	5	4	28	28	24	0	0	One female died 73 days after mating, and two were sterile.
996	1st	301	3	18	146	144	105	76	72	
	2nd	218	4	8	46	42	40	15	37	
	3rd	153	2	6	43	41	34	12	35	
	4th	244	3	3	20	20	17	17	100	One female developed tumor, was eliminated before mating.
	5th	170	4	2	8	8	8	0	0	One female, sterile, died 60 days after mating.
997	1st	311	3	18	150	150	105	102	97	
	2nd	235	4	17	144	141	95	57	60	
	3rd	154	6	12	85	73	61	33	54	Two females were sterile.
	4th	341	3	12	73	68	56	42	75	One female, one male died three months before completion of experiment.
	5th	187	4	9	49	41	40	21	52	

TABLE II (continued)

998	1st	285	2	9	85	85	53	41	77	Two females completely failed in growth 60 days before termination of experiment. One of these died.
	2nd	229	4	11	94	92	62	40	64	
	3rd	162	4	12	78	77	67	33	49	
	4th	244	4	9	45	36	32	22	69	
	5th	151	3	3	19	13	13	6	46	
999	1st	285	3	17	147	147	100	87	87	Two females sterile. Out of five young reared, only one was female.
	2nd	235	5	17	130	118	117	51	44	
	3rd	159	4	8	44	19	19	9	47	
	4th	77	4	2	11	11	10	5	50	
	5th	49	1	0	0	0	0	0	0	

supplemented by butter fat, as evidenced in studies of lactation conducted during a period of several years. An examination of Tables II and III shows that for every increase of 2 per cent of butter fat there is a corresponding increase in 12 per cent of young successfully weaned. It is also quite apparent that ration 997 produced optimum results in both fertility and lactation. The greater number of young born on ration 995 than on ration 996 I am unable to explain. The higher percentage of sterility and dead young born on ration 995 compared with ration 996, however, is evident.

On neither ration 995 nor 996 were young reared in the fifth generation and the increase of only 2 per cent butter fat changed the lactation index in the fifth generation from entire failure to 49 per cent efficiency, as indicated by the results secured on rations 996 and 997 (Table II).

It is also evident that rations 998 and 999, which are duplicates of Sherman and Muhlfeld's whole milk powder diet B (4), containing a total of 9.17 per cent milk fat, did not prove as efficient as ration 997, containing only about 0.4 per cent additional milk fat. Table III shows that the increase of lactation efficiency on rations 996 and 997, compared with results secured on ration 995, follows a curve of arithmetical progression, *i.e.*, for every 2 per cent additional amount of butter fat there is a corresponding increase of 12 per cent in rearing of young; therefore, 0.4 per cent less butter fat should have produced a 2.4 per cent less efficient lactation record



TABLE III

SUMMARY OF FERTILITY AND LACTATION RECORDS OF ANIMALS ON RATIONS 995 TO 999 INCLUSIVE COVERING FIVE GENERATIONS

Ration	Total reproduction period (days)	Females	Sterile females	Litters	Young born	Young born alive	Young allowed to be reared	Young weaned	
								No.	per cent
995	967	18	4	46	380	362	257	120	47
996	1,086	16	1	37	263	255	204	120	59
997	1,228	20	2	68	501	473	357	255	71
998	1,071	17	0	44	321	303	227	142	62
999	805	17	2	44	332	295	236	152	64

on rations 998 and 999 than on ration 997. Actually, there was a decrease of 9 per cent, or for every 65.4 young expected to be weaned out of 100 there was a loss of 5.6 per cent efficiency. This slight difference in lactation efficiency, however, may not at all be due to destruction of vitamin A in whole milk powder during the process of drying, but may be due to biochemical changes produced in the whole milk powder during storage of that material in summer months in our laboratory.

An examination of all the milk powder diets used in this investigation reveals that none can be considered optimum for lactation. The fortification of the rations in the fifth generation with vitamin E furnished by wheat oil did not improve the lactation efficiency index. On the other hand in a good many instances we have observed polyneuritis in failing nursing young which readily responded to vitamin B therapy, the vitamin being furnished to the nurslings in the form of concentrated extracts from yeast or rice polishings. It is, therefore, concluded that vitamin B is one of the limiting factors in such milk diets. That milk is deficient in vitamin B for lactation was demonstrated in 1924 (5), since rations composed of 50 per cent skimmed milk powder had to be supplemented with a brewer's yeast concentrate, in order to make them adequate for rearing of young. The irregular and conflicting results recently reported by Daniels, Jordan and Hutton (6) on the potency of milk in vitamin B for lactation are due to the failure of these investigators to deplete the lactating mothers of vitamin reserves, and also to the failure to use an experimental maternal diet satisfactory in every respect with the exception of vitamin B. For the same reasons the conclusions of Daniels, Giddings and Jordan (7) on the destruction of vitamin B in evaporated milk also are open to criticism.

Although we find the Sherman and Muhlfeld diet B (our rations Nos.

998 and 999) unsuitable for raising a vigorous rat colony, which conclusion is in agreement with the recent report of Smith and Anderson (8) we consider that diet, supplemented with fresh vegetables, preferable for raising animals preparatory to studies on vitamins A or D, since it allows less storage of such fat-soluble vitamins.

### SUMMARY

1. The importance of small variations in the amount of milk fat in fertility, and particularly in lactation, is demonstrated.

2. One of the limiting factors in all the milk diets studied from the standpoint of lactation is vitamin B.

### BIBLIOGRAPHY

1. Sure, B., *Proc. Soc. Exp. Biol. and Med.*, 1929, XXVII, 148.
2. Associates of Rogers, *Fundamentals of Dairy Science*, 1928, 34.
3. *Ibid.*, 1928, 32.
4. Sherman, H. C., and Muhlfeld, M., *Jour. Biol. Chem.*, 1922, LIII, 41.
5. Sure, B., *Jour. Biol. Chem.*, 1924, LXII, 371.
6. Daniels, A. L., Jordan, D., and Hutton, M. K., *This Jour.*, 1929, II, 19.
7. Daniels, A. L., Giddings, M. L., and Jordan, D., *This Jour.*, 1929, I, 445.
8. Smith, A. H., and Anderson, W. E., *Science*, 1929, LXX, 99.



# THE COMPARATIVE VALUE OF DIFFERENT FOOD PROTEINS FOR REPRODUCTION AND LACTATION IN THE RAT\*

## I. BEEF MUSCLE, LIVER AND KIDNEY

BY MARY M. CLAYTON

With the assistance of Marian J. Cummings  
(*From the Department of Vital Economics, University  
of Rochester, Rochester, N. Y.*)

Received for Publication—October 16, 1929

THE science of nutrition has been greatly advanced during the last two decades through an increase in knowledge of the unequal nutritional values of amino acids, the rôle of mineral salts in vital processes, and the indispensability of vitamins. The biological method of experimentation was applied to the study of foods at first only in so far as they concerned growth and maintenance. It was McCollum (1915) who first suggested the importance of such study for reproduction and lactation. "Only when normal reproduction and rearing of young is repeated at normal intervals can a ration be said to be physiologically sufficient." Since that time much effort in this direction has revealed that for the proper performance of these functions an animal has very definite qualitative and quantitative needs in the way of dietary constituents. These needs, as far as they were known, were stated clearly by Simmonds (1924). If these needs are not met, young may never be born, or if they are born, their lives may be brief because of the lack of some necessary constituents for the formation of normal milk. Sometimes the young are normal when born and remain so until weaned, but only at the expense of the mother who is found in an emaciated condition, due to the vicarious supply from her own tissues of certain substances indispensable for milk production. Or, finally, the young may be born and reared normally but prove to be incapable of procreation.

The acquisition of data is made the more difficult by the larger number of variables involved, most of them of unknown magnitude. Before a qualitative or quantitative inadequacy in one dietary constituent can be accepted as proved, the "law of the minimum" for all other dietary requisites must first be complied with. A recognition of the indispensable food entities has been and is being achieved only by the same biological

\* The material in this and a following paper was submitted to the University of Rochester in partial fulfillment of the requirements for the degree Doctor of Philosophy. The experimental work was done under a fellowship granted by the National Live Stock and Meat Board.

method, and it is therefore not surprising that often purely accidental variations in diet have led to the discovery of hitherto unsuspected essentials. Vitamin E came into being as the proper interpretation of the results of a chance dietary variation (Evans, 1922, 1923).

The need of increased amounts of vitamins for reproduction and still larger amounts for lactation over the maintenance requirements has been demonstrated (Sure, 1926, 1928). It is well known that a protein of good quality is necessary for normal reproduction and lactation, but the quantity required has not been exactly determined. McCollum (1915) first emphasized the importance of mineral salts for normal procreation. Many workers in this field have attempted to supply all of the presumably necessary elements but much remains to be learned about the actual salt requirements with different kinds of diets, the actual needs of the body for certain of the rarer elements, and the catalytic, or other, effects which certain of them, such as iron and copper, have upon the body processes.

The purpose of the experiments described in this and the following paper was to compare beef muscle, liver, kidney, milk and egg for reproduction and lactation in the rat.

### HISTORICAL

The use of an exclusive diet of muscle or gland tissue has always been followed by nutritive failure. Of C. Watson's (1906) 14 young rats on ox flesh, 5 died within four months, the others were undersized and sterile. Partly grown rats on this food became pregnant but failed to rear their young. The similar experiments of B. P. Watson (1907) in which partly grown rats were subjected to short periods of meat feedings, resulted in poorly nourished litters and subnormal weights of the mammae of the mothers.

Later work on muscle tissue by McCollum (1921) and Osborne and Mendel (1917, 1918) revealed that this is low in vitamin B and lacking in vitamin A and calcium. When fed on a 20 per cent level with mineral and vitamin A supplements, animals did not attain normal growth, and reproduction and lactation were unsuccessful. This suggests an inferior quality of protein and also inadequate vitamin E. The contrary observations of Evans and Burr (1927) who, to be sure, used a different technique, are illustrative of the influence of unknown variables.

Liver tissue, according to the same groups of workers, contains considerable amounts of vitamins A and B, but lacks calcium. The protein appears to be of better quality than that in muscle tissue. Kidney tissue is rather more complete. The protein is of excellent quality and the vitamin A content high. Vitamin B appears to be present in smaller amount than in liver. The content of calcium is low.

The biological values of the proteins of muscle, liver and kidney on an 8 per cent level, as determined by Mitchell (1926, 1926-1927), were 69, 77, and 77 per cent respectively. Fed on a 15 per cent level with necessary non-protein supplements, Hoagland and Snider (1926) found little difference between dried ox muscle and liver when measured by growth. The results of Hartwell (1921, 1924) were at first baffling but her conclusions were confirmed by Hassan and Drummond (1927) with special emphasis upon the need of increasing amounts of the heat-stable factor of vitamin B when protein intake is increased.

## EXPERIMENTAL

*Animals.* The animals, obtained either from our own stock or from the Albino Supply Company in Philadelphia, usually weighed from 45 to 65 grams when put on experiment, seldom more. Individual charts were made for each animal. These contained the reproductive history as well as the growth curve. Females were weighed regularly once a week, pregnant animals every day. Litters were weighed once a week and males once in two weeks. The behavior of each litter was carefully noted from birth until death or weaning time. This involved daily observation of each mother and her young. During the course of the whole experiment, charts were made for 648 animals and records were kept of 711 litters.

*Rations.* In the first comparative rations it was thought best to supply known sources of all the food constituents which were considered to be necessary for reproduction and lactation in the rat. The protein was supplied entirely by the meat, with the exception of the small amount present in the yeast which was used as a source of vitamin B. The meat was also the only source of vitamin E. Fat, when needed, was supplied by lard, carbohydrate by cornstarch, and vitamins A and D by cod liver oil. The salt mixture used was that of Osborne and Mendel (1917). The protein and vitamin E in the meats thus became the only known variables.

In the earlier series of experiments, the meats were fed in cooked, dried form. The beef round, liver, and kidney were prepared as follows: After the removal of all visible fat they were cooked in steam in an autoclave at 10 pounds pressure for one hour. They were then ground in a meat grinder. The juices collected in the cooking vessels were evaporated to a small volume on a water bath and added to the meat which had been partially dried at a low temperature (not over 40 degrees C). The drying was completed at the same temperature and the meats were then ground to a fine powder.

The gross chemical composition of the dried meats was determined by analyses of several lots and was as follows:

	Protein	Fat	Ash
Beef round	74.01	11.40	3.69
Beef liver	62.00	10.25	4.71
Beef kidney	70.00	11.66	5.12

## SERIES I—MODERATE PROTEIN, HIGH FAT

The first series of rations was planned to contain 15 per cent protein, 20 per cent fat, and 5 per cent ash and on the basis of the above analysis they were constituted as follows:

COMPOSITION OF RATIONS IN SERIES I  
(15% protein, 20% fat, 5% ash)

Ration No.	Product (powdered)	Starch	Lard	Salts	Yeast	C.l.o.
120	Round	20.3	52.5	15.7	4.5	5.0
130	Liver	24.2	49.3	15.5	4.0	5.0
140	Kidney	21.4	52.1	15.5	4.0	5.0

The 15 per cent protein level was chosen for a beginning because this was the lowest level at which McCollum (1921) obtained successful results in reproduction and lactation on meat diets. His diets differed from ours, however, in several respects, the chief difference being that they contained 3 per cent of butterfat, no yeast, and no lard.

In preparing the diets of series I, all of the ingredients were thoroughly mixed together in 1 or 2 kilogram lots and placed in open tin cans. As long as the food lasted it was exposed to the temperature and air of the rat room. As a rule, however, fresh food was made up once a week or oftener. No records were kept of food consumption. Food was always available to the animals.

*Beef Round, Ration 120.* The 8 females and 6 males on this ration grew normally, or better, without exception, but reproduction was practically a failure. Up to 125 days, only two females had borne litters. Soon after this 3 per cent of wheat germ oil was added to the ration as a source of vitamin E. All of the females then had litters but only 3 had any success in lactation. One female with lung disease was anesthetized; the other 4 were given an alcoholic extract of wheat embryo<sup>1</sup> to see whether lactation would be improved. It was improved in three of them. The results were as follows:

	No. Born	No. Lived	% Lived
After oil	68	13	19.1
After oil & extract	39	25	64.0

The one female which did not show improved lactation was found to have badly diseased lungs.

The 6 males all received the 3 per cent oil addition. Only one appeared to be infertile and it showed testes weights 49 per cent of normal.

Ten second generation females and 2 males were kept on the ration with the oil addition. Their growth was normal or above. However, only 8 females had litters and only 3 had any success in lactation. There were a great many cases of diseased lungs. The males were both fertile.

*Beef Liver, Ration 130.* Two lots of animals were placed on this ration, and as the results differed in some respects, the two groups will be considered separately.

In the first group of 5 females and 4 males growth was normal or better and in the beginning all animals were fertile. After 175 days, however, fertility ceased, showing a deficiency of vitamin E. There was some success in lactation, 39 per cent of the young being weaned, but some were underweight.

<sup>1</sup> The extract was made as follows: Two liters of 70 per cent alcohol were added to 400 grams of ether-extracted wheat embryo in a Florence flask. The flask was stoppered and thoroughly shaken several times a day for five days. The extract was then filtered off with suction and evaporated to about one-half its volume by use of an electric fan at room temperature. One-half the total volume was used in one kilo of ration, the extract being poured on starch and allowed to evaporate to dryness before the other ingredients were added. This extract was used as an additional source of the vitamin B complex, or perhaps of a new accessory of water-soluble nature, mentioned by Evans, (1924).

At autopsy the parents showed considerable lung disease and a peculiar form of fatty degeneration of the liver. The tissue had a yellow mottled appearance and was tough and fibrous. Three of the males showed degeneration of the testes. The second generation animals were all sterile but showed no apparent liver changes.

The second group of animals showed better growth than the first and fertility was maintained a little longer, but lactation was not so successful, only 8 per cent of the young surviving. At autopsy the animals showed considerable lung disease but the abnormal liver condition was not found. One male and one female of the second generation were kept on the ration and both were sterile.

*Beef Kidney, Ration 140.* The 8 females and 5 males on this ration showed a growth rate which was normal or above. At the end of 125 days only 4 of the females had reproduced and none of the young had been raised. Since there was an apparent deficiency of vitamin E, 2 per cent of wheat germ oil was added to the ration. Five of the females then had litters but 2 remained sterile and another became so. The reason for this failure to respond to wheat germ oil was probably the fact that the females were mated with a male which was later found to be sterile. One of them was also found to have ovarian disease. There was some success in lactation following the oil addition, 41.7 per cent of the young being weaned.

Eight second generation females and 4 males were kept on the ration with the 2 per cent oil addition. All but one female grew very well but reproduction was not uniformly successful. Three females were sterile but this may have been due to the fact that they were mated with males whose fertility was questionable.

Lactation was a complete failure in two animals but fair in others, 25.7 per cent of the young born surviving and making unusually good growth.

Three females and one male from the third generation were kept on the same ration but all were sterile.

### DISCUSSION OF RATIONS IN SERIES I

Of the meat rations, that containing liver was easily the best so far as reproduction was concerned, but even here it failed before the end of the first generation. Since the addition of wheat germ oil greatly improved the reproductive efficiency of the muscle and kidney rations, it is reasonable to conclude that vitamin E was the limiting factor in the liver ration also. Practically the same amount of lard was present in each of the three rations, hence the superiority of liver must have been due to some quality of the liver itself. The influence of lard will be discussed later.

None of the rations in this series was very successful for lactation. Liver at first appeared to be superior, as 39 per cent of the young of the first group were weaned. In the second group, however, only 8 per cent lived.

The addition of the alcoholic extract of ether-extracted wheat germ apparently improved lactation on the muscle diet and from the work of Evans and Burr (1928) and Sure (1927, 1928) it seems probable that the effective substance in the extract was vitamin B, particularly the heat-labile fraction, which was described by Smith and Hendrick (1926), Goldberger (1926), and others and which eventually will probably be called vitamin B or B<sub>1</sub>.<sup>2</sup> Wheat embryo has been found by Chick and Roscoe

<sup>2</sup> Following the recommendation of the Committee on Vitamin B terminology appointed by the American Society of Biological Chemists (*Science*, 1929, LXIX, 276).



(1927) to be rich in this heat-labile factor and poor in the heat-stable factor (G or B<sub>2</sub>). Yeast contains both factors, but Evans and Burr (1928) have found that it requires 15 per cent of brewer's yeast to provide enough of each for lactation. Liver and kidney are known to contain more of the vitamin B complex than muscle, and it is probable that the method of preparing the meats in dry form greatly reduced the amount of vitamin B (antineuritic) in muscle so that the 5 per cent of yeast was no longer adequate. Hence the effectiveness of the wheat embryo extract. Unfortunately it was not used as a supplement to the liver and kidney rations, and these tissues as well as many other foods must be restudied in view of the composite nature of vitamin B.

#### EFFECT OF USE OF SPECIAL SALT MIXTURE IN LIVER RATION 130

The effectiveness of certain mineral additions to milk diets in promoting reproduction and lactation (Daniels, 1925) suggested the substitution of a special salt mixture<sup>a</sup> containing these minerals in the best ration of this series (liver).

*Beef Liver, Ration 130S.* The liver diet was not improved in any way by the special salt mixture. Six females and one male were placed on the ration. The 3 females which were first mated with this male had one litter each but the others which were mated with second generation males from ration 130 were sterile. Lactation was a failure.

#### SERIES II—HIGH PROTEIN, HIGH FAT

The rations in Series II were made to contain 20 per cent protein with the idea that by increasing this constituent and at the same time the other constituents of the meats, the deficiencies apparent in Series I might be overcome. The rations were as follows:

<sup>a</sup> The salt mixture, modified from Osborne and Mendel (1918) comprised the following:

	gms.		gms.
CaCO <sub>3</sub>	539.2	Citric acid (dry)	406
MgCO <sub>3</sub>	96.8	Iron citrate	25.36
Na <sub>2</sub> CO <sub>3</sub>	136.8	KI	1.4
K <sub>2</sub> CO <sub>3</sub> 1-1/2 H <sub>2</sub> O	675.6	MnSO <sub>4</sub> ·H <sub>2</sub> O	8.7
85% H <sub>3</sub> PO <sub>4</sub>	284 cc.	NaF	8.5
Conc. HCl	504 cc.	Alum	4.2
Conc. H <sub>2</sub> SO <sub>4</sub>	21 cc.	Na <sub>2</sub> SiO <sub>3</sub> ·H <sub>2</sub> O	25.0

COMPOSITION OF RATIONS IN SERIES II  
(20% protein, 20% fat, 5% ash)

Ration No.	Product (powdered)	Starch	Lard	Salts	Yeast	C.l.o.
121	Round 27.0	46.6	14.9	4.5	5.0	2.0
131	Liver 32.2	42.1	14.7	4.0	5.0	2.0
141	Kidney 28.0	46.3	14.7	4.0	5.0	2.0

*Beef Round, Ration 121.* The 8 females and 5 males on this ration showed growth which was above normal. The females all had litters but in only one case did fertility continue after the animals were 175 days of age. Lactation was only slightly successful, 10.8 per cent of the young being reared. Two of the males were discarded early in life. Of the other 3, two were sterile and one was questionable.

Six females and 2 males were carried through the second generation. Growth continued to be excellent but reproduction was a complete failure.

It is therefore evident that the presence of enough muscle tissue to make 20 per cent protein provided for some success in reproduction and lactation in the first generation but none in the second. Vitamin E was deficient to judge from the improvement in reproduction which was obtained when wheat germ oil was added to ration 120 containing 15 per cent of muscle protein. Vitamin B was but slightly increased in ration 121.

*Beef Liver, Ration 131.* The seven females and 6 males placed on this ration all showed a growth considerably above normal. As in the case of the ration containing 15 per cent liver protein the animals were at first fertile. However, after the females were 175 days of age no litters were born. Lactation was improved to some extent, 50.6 per cent of the young surviving.

The ten females and 4 males of the second generation were all sterile.

Increasing the protein to 20 per cent thus apparently had little effect in improving reproduction, but caused some improvement in lactation, suggesting an increased vitamin B supply.

*Beef Kidney, Ration 141.* Nine females and 5 males were placed on this ration. Growth was above normal but continued reproduction was not secured. At the age of 175 days 2 per cent of wheat germ oil was added to the ration, but with little effect, as the males had already become sterile. The few litters which were born were the result of mating with colony males. There was no success in lactation before the addition of wheat germ oil, but of the ten young born afterward, five lived. These made exceptionally good growth.

No second generation animals were continued on the ration.

Increasing the protein content of this kidney ration caused a slight improvement in reproduction but none in lactation.

## DISCUSSION OF RATIONS IN SERIES II

(See Table I, page 500)

All of the animals on the Series II rations were obviously suffering from a deficiency in vitamin E and it is unfortunate that in several instances this was supplied too late in the life of the male animals. Their sterility had become incurable, as happens after about 150 days, and sometimes earlier, according to Mattill (1924) and Evans and Burr (1927).

As a result of recent experimentation by a number of workers it seems very probable that the deficiency in vitamin E in the rations of both Series I and II may be partly accounted for by the presence of the unsaturated animal fats, lard and cod liver oil. Anderegg and Nelson (1926) dem-

onstrated that with the development of rancidity, fats have a destructive effect on vitamins which is associated with processes of oxidation. Ferrous sulphate also has a catalytic effect on oxidative processes, as was demonstrated by Simmonds, Becker, and McCollum (1927). The function of wheat germ oil in a ration is apparently that of an antioxidant as well as a source of vitamin E. Mattill (1927) thinks that a possible explanation for this protective action of wheat germ oil is found in the experiments of Holm (1927) to the effect that the OH group has a very powerful retarding action upon oxidation. It thus appears that the degree of success in reproduction on a ration may be a measure of its content of antioxidants, when such a ration also contains auto-oxidizable fats.

Since the 5 per cent increase in muscle protein was most effective in improving reproduction, it might be argued that muscle contains more antioxidant than kidney or liver, a statement which agrees with Evans' observations on the distribution of vitamin E in these same tissues.

As regards lactation, liver took first place, followed afar by muscle and kidney. The kidney ration, like that in Series I, was of no value for lactation until after the addition of 2 per cent of wheat germ oil. It would seem that dried beef kidney may contain a considerable amount of the heat-labile vitamin B. In the presence of sufficient vitamin E, this becomes available for lactation.

The protein of kidney is apparently of unusually good quality for growth. The young rats on ration 141 were of very large size at weaning time.

### SERIES III—MEDIUM PROTEIN, LOW FAT

With the object of further testing the deleterious effect of lard in rat diets, Series III rations were prepared. They contained, as shown below, a quantity of fat (10 per cent) more nearly like that usually present in human dietaries and slightly more than half of this was lard.

COMPOSITION OF RATIONS IN SERIES III  
(15% protein, 10% fat, 5% ash)

Ration No.	Product (powdered)	Starch	Lard	Salts	Yeast	C.I.o.	
123	Round	20.3	62.5	5.7	4.5	5.0	2.0
133	Liver	24.2	59.3	5.5	4.0	5.0	2.0
143	Kidney	21.4	62.1	5.5	4.0	5.0	2.0

*Rations 123, 133, 143.* It was expected that these three rations would at least equal the three corresponding rations in Series I. It is rather difficult to make a direct comparison except in the case of the liver rations, owing to the fact that additions of wheat germ oil were made so early in the career of the animals on rations 120 (round) and 140 (kidney). It is possible, however, to compare the results of the two series up to the time the animals were 150 days of age, as follows:

	Ration	No. born per Female	Per cent which lived
Round	120	1.4	0
	123	4.4	0
Liver	130 1st lot	6.0	50
	130 2nd lot	9.3	10.7
	133	2.4	0
Kidney	140	2.3	0
	143	4.6	0

There was a slight improvement in reproduction on round and kidney following the decrease in fat. Results on liver were not so good and there seems to be no way of explaining this fact. Lactation was a complete failure in the case of all three rations. One may conclude therefore that in a ration containing 15 per cent of protein and 10 per cent of fat, part in the form of lard and cod liver oil, neither beef round, nor liver nor kidney is adequate for reproduction and lactation.

#### SERIES IV—HIGH PROTEIN, NO ADDED LARD

The results secured in the rations of the first three series naturally led to a fourth containing 20 per cent of protein and no added fat except 2 per cent of cod liver oil to supply vitamins A and D.

##### COMPOSITION OF RATIONS IN SERIES IV (20% protein, no lard, 5% ash)

Ration No.	Product (powdered)	Starch	Salts	Yeast	C.l.o.*	
124	Round	27.0	61.5	4.5	5.0	2.0
134	Liver	32.2	56.8	4.0	5.0	2.0
144	Kidney	28.0	61.0	4.0	5.0	2.0

\* Added daily after 135 days.

*Beef Round, Ration 124.* The 8 females and 3 males on the ration all showed growth which was normal or above. Before the cod liver oil was added daily, only 4 of the females had litters. Afterward all were fertile. Of the young born, 16.5 per cent lived. The 3 males all remained fertile.

Five second generation females and 2 males were kept on the ration. Growth was normal or above and fertility continued. None of the 3 females had litters up to the age of 125 days, but all were fertile later. Lactation was improved, 51 per cent of the young born now surviving.

Three females and one male from the third generation were kept on the ration. Their growth was normal, but owing to shortness of time they were anesthetized at 155 days of age, before reproduction had begun.

*Beef Liver, Ration 134.* Most of the 9 females and 3 males on this ration showed growth which was above normal. Two of the females were infertile and one died at the birth of her first litter before the cod liver oil was added daily. Afterward, the 8 living females all had litters. One, which showed poor fertility, had an infected uterus. Sixteen and one-tenth per cent of the young born were weaned. The males all remained fertile.

Six second-generation females and three males were kept on the ration. Growth was not quite so good and only three females were fertile. Of the young born, 27.7 per cent were weaned. At autopsy two of those which were infertile showed no scars on the uterus, proving that they had never been pregnant. Two of the females also showed slight indication of a fatty degeneration of the liver, similar to that which was noted in the first group of animals on ration 130.

*Beef Kidney, Ration 144.* Growth on this ration was above normal in most cases. Three of the 8 females were sterile before the cod liver oil was added daily, but afterward all were fertile. Reproduction was not so good, however, as on rations 124 and 134, and only 6.8 per cent of the young born were weaned. The animals showed considerable lung disease at autopsy, which may partly account for the poor results. The 3 males were all fertile.

Five females and 2 males from the second generation were kept on the ration. Only one female was fertile but embryos were present in 3 others at autopsy. The one fertile female raised one litter of seven and lost one. The males were both fertile.

One male and one female were kept from the third generation. The female was anesthetized at the age of 87 days but showed 9 half-grown embryos.

#### DISCUSSION OF RATIONS IN SERIES IV

The marked improvement in reproduction following the removal of lard from the rations and the addition of the cod liver oil daily is plainly shown in Table I, in which the results on the rations of Series II and IV

TABLE I  
COMPARISON OF RESULTS IN SERIES II AND IV

Ration	No. females	Av. age at birth first litter days	Av. No. lit. per female to 255 days	Av. No. born per female	Per cent that lived	Remarks
121, Round, lard + c.l.o	8	112	2.0	11.5	10.8	2nd generation sterile
124, Round, no lard, c.l.o after 135 days	8	121	2.9	23.0	11.8	2nd generation fertile
121, Liver, lard + c.l.o	7	100	1.9	11.3	50.6	2nd generation sterile
134, Liver, no lard, c.l.o daily after 135 days	9	117	2.9	19.1	12.2	2nd generation 50% fertile
141, Kidney, lard + c.l.o	8	121	1.4	3.1	0.0	No 2nd generation
144, Kidney, no lard, c.l.o. daily after 135 days	8	149	2.3	14.5	7.7	2nd generation partly fertile

are compared. The contrast in results in the second generation is particularly striking. Lactation was also improved in the Series IV rations containing round and kidney but not in the one containing liver.

#### SERIES V—HIGH PROTEIN, TEST FOR VITAMIN B

The rations of Series V were designed to indicate the relative vitamin B content of the dry meats used, and since muscle is already known to be a poor source of vitamin B (Osborne, 1917, 1918) it was omitted from the series, whose composition was the following:

COMPOSITION OF RATIONS IN SERIES V  
(20% protein, no lard, no yeast, 5% ash)

Ration No.	Product (powdered)	Starch	Salts	C.I.o. *
135	Liver 32.2	61.8	4.0	2.0
145	Kidney 28.0	66.0	4.0	2.0

\* Added daily after 125 days

*Beef Liver, Ration 135.* The 8 females and 3 males all showed exceptionally good growth. They were all fertile and the females had repeated litters but very little success in lactation. Only 2.6 per cent of the young born were weaned. Two of the animals showed badly diseased lungs at autopsy and 2 had inflamed intestines.

Of the 3 second generation females, only one was fertile and this animal had some success in lactation. One of the 2 second generation males was sterile, which accounts for the failure of the other 2 females to reproduce.

*Beef Kidney, Ration 145.* Growth was not quite so good as on the liver ration. The 8 females were all fertile but nearly all had their first litters late and 3 were infertile late in life. Only 4.8 per cent of the young born survived. The two second generation females and one male were all fertile.

### DISCUSSION OF RATIONS IN SERIES V

It would appear that dried kidney contains a little less vitamin B than dried liver. The fact that all of the animals made normal growth shows, however, that liver and kidney both contain sufficient vitamin B for normal growth when fed at a 20 per cent level.

Since the requirements for vitamin B are so much greater for lactation than for growth, the effect of removing the yeast from rations 134 and 144 is evident in the animals on series V rations.

Rations 134, 135, 144 and 145 are compared in Table II. In the Series

TABLE II  
RESULTS WITH AND WITHOUT YEAST

Ration 20% prot. no lard, c.I.o. daily after 135 days	No. females	Av. age at birth 1st lit. days	Av. No. lit. per female to 255 days	Av. No. born per female	Per cent that lived	Remarks
134, 50% yeast	9	117	2.9	19.1	12.2	2nd generation 50% fertile
135, No yeast	8	92	4.3	28.5	1.3	2nd generation partly fertile
144, 50% yeast	8	149	2.3	14.5	7.7	2nd generation partly fertile
145, No yeast	8	185	2.5	19.0	4.6	2nd generation fertile

V rations there is an increase in the number of young born but a distinct decrease in the per cent of young which were weaned.

## SERIES VI—HIGH PROTEIN, TEST FOR VITAMIN A

The rations of series VI were planned for two reasons. First, it seemed desirable to make a comparison of the protein foods for vitamin A content. Second, since the addition of the cod liver oil daily to the rations of Series IV and V had such a beneficial effect on reproduction, it seemed desirable to omit it entirely, relying on the protein foods themselves for vitamin A and radiating<sup>4</sup> the animals to provide vitamin D. Since beef muscle is known to be low in vitamin A that meat was omitted from the series. The rations were as follows:

COMPOSITION OF RATIONS IN SERIES VI  
(20% protein, no lard, no c.l.o., 5% ash)

Ration No.	Product (powdered)	Starch	Salts	Yeast
136	Liver 32.2	58.8	4.0	5.0
146	Kidney 28.0	63.0	4.0	5.0

The animals in this series showed unusual success in reproduction but not in lactation. In the attempt to determine the missing factor or factors, an addition of 2 per cent of wheat germ oil was first made to the rations at the time the animals were about 175 days of age. A little later 3 per cent more of yeast was added, making a total of 8 per cent. The results of these modifications will be taken up in the discussion of each ration.

*Beef Liver, Ration 136.* The 9 females and 3 males on this ration nearly all showed growth which was above normal. Three of the females had no litters before the oil addition and one was sterile throughout life. Only one litter of two was raised before oil. After oil and yeast the same female raised a litter of seven. Oil alone cannot be said to have improved lactation. After the yeast addition, 3 females together raised 11 young. Two of the males were fertile but one was questionable. At autopsy 2 of the animals were shown to have slight fatty degeneration of the liver, similar to that found in the first lot on ration 130. Three had badly diseased lungs.

The two females and one male from the second generation showed very good growth and all were fertile. One female raised a litter of 4 following oil and yeast.

*Beef Kidney, Ration 146.* The 8 females and 3 males on this ration all showed growth above normal. All but 2 of the females were fertile before the oil addition. One of the 2 which were sterile before oil, had lung disease and was anesthetized. The other had a litter following the oil. The remaining 6 had repeated litters of large size but only 2 raised young. There was no improvement after oil alone, but after oil plus yeast, 2 females together raised 15 young. Results might have been better had there not been so much lung disease.

## DISCUSSION OF RATIONS IN SERIES VI

Interpretation of results on the rations of this series is complicated by the fact that infections were so prevalent among the animals. This susceptibility to disease has been noted by a number of workers to exist among animals deprived of adequate amounts of vitamin A (Steenbock,

<sup>4</sup> The animals were radiated twice a week for 3 minutes at a distance of three feet. The lamp used was a Hanovia Quartz Mercury Arc.

1923; Sherman, 1925). One fact is very evident, however, that by omitting cod liver oil entirely, ration 146 was made better for reproduction than ration 144, which contained cod liver oil. The improvement was noted especially in the average number born per female and in the average number of litters per female up to the age of 140 days, as shown in Table III.

TABLE III  
RESULTS WITH AND WITHOUT COD LIVER OIL

Ration	No. females	Av. age at birth 1st lit. days	Av. No. lit. per female to 140 days	Av. size litter	Av. No. born per female	Per cent that lived
134, Liver, +c.l.o.	9	117	1.20	6.4	7.7	0.0
136, Liver, no c.l.o.	9	114	.78	8.0	6.2	3.6
144, Kidney, +c.l.o.	8	149	.87	6.9	6.0	0.0
146, Kidney, no c.l.o.	8	106	1.40	9.1	12.5	8.0

The animals on ration 136 were no more fertile than those on 134. The comparison can be carried only up to the time the animals were 140 days of age, on account of the fact that wheat germ oil was added to the rations of Series VI when the animals were about that age.

It was rather surprising that reproduction was better on ration 146 than on 136 and there seems to be no explanation for the fact except that kidney protein is superior to liver protein.

Lactation was practically a failure on the rations of this series. The reason for this is a matter for speculation. The animals seemed to have enough vitamin A for growth and reproduction but it may be that an additional supply is needed for lactation, as is true of vitamin B, especially the heat-labile fraction. The fact that so many of the females had diseased lungs may also account for the poor lactation. Table III really gives a wrong impression as to the success in lactation on rations 134 and 144, since the figures were compiled before the females had borne very many litters. As will be seen from Table III, there was some success in lactation on these rations. However, that function was not nearly so good as it should have been, and it is very probable that those rations would have been improved for lactation by the addition of more yeast, just as the Series VI rations were.

If freedom from infection on a ration is an indication of the presence of adequate amounts of vitamin A, it can be said that ration 136 (liver) appears to have contained a little more than the other rations in the series. This agrees with the work of Sherman and Boynton (1925). Those



workers state that rat liver contains 200 to 400 times as much vitamin A as rat muscle.

### SERIES VII—RAW PROTEIN, NO ADDED LARD

Finally, in order to determine whether cooking has any detrimental effect on the nutritive value of the meats, it was thought desirable to try a series of rations containing them in the raw condition. In planning the rations it was decided that as much of the raw meats should be used as would be necessary to provide 15 per cent of protein if they were in the dry form. The amounts of the other constituents were then calculated by assuming that the meat was dried. The rations were as follows:

COMPOSITION OF RATIONS IN SERIES VII  
(15% uncooked protein, no lard, 5% ash)

Ration No.	Raw protein		Starch	Salts	Yeast	C.I.o*
	Food	gms.	gms.	gms.	gms.	gms.
127	Round	71.4	68.2	4.5	5.0	2.0
137	Liver	68.2	64.8	4.0	5.0	2.0
147	Kidney	87.2	67.6	4.0	5.0	2.0

\* Daily

In feeding these rations, the raw meats plus the cod liver oil were added daily to the dry basal mixture, which was made up in large quantities. The meats were first carefully freed from visible fat and ground finely in a meat grinder.

*Beef Round, Ration 127.* All but one of the 8 females and all of the 3 males on this ration showed normal growth up to 100 days of age, but after that time several showed a decline. Reproduction records were poor. Four of the females were infertile, one had 2 litters, and the other 3 had one litter each. These results in the females may have been partly due to the males, 2 of which were apparently infertile, although their testes were 85 and 88 per cent of the normal weight. Many of the animals showed lung disease.

*Beef Liver, Ration 137.* Growth on this ration was normal, or above, with one exception. Two of the 8 females were infertile. Both of these showed badly diseased lungs and one also had a diseased liver and uterus. In fact, all but one of the animals on this ration developed some lung disease. Two of the 3 males proved to be infertile, which partially accounts for the poor fertility of the females. The growth of the one fertile male was unusually good and this animal was free from infections. Lactation was fair, 30 per cent of the 56 young surviving.

Two females and one male from the second generation were kept on the ration. Growth was above normal but the animals had to be discarded before the reproductive period had begun.

*Beef Kidney, Ration 147.* The 9 females and 3 males on this ration all showed excellent growth. The females were all fertile but the records of three were poor, due to the fact that they were mated with the one male which proved to be infertile. A total of 110 young were born, of which 33 per cent were weaned.

Five females and 3 males from the second generation were kept on the ration. Two of the females had litters and 2 of the males were shown to be fertile. The others were anesthetized while still too young for reproduction.

## DISCUSSION OF RATINGS IN SERIES VII

The poor results on raw beef muscle were probably partly due to the large amount of tough connective tissue, which caused poor assimilation of the meat. At least this explanation is suggested by the work of Mitchell (1927), who found that the feeding of connective tissue with the muscle tissue of pork tenderloin caused a depression in the biological value of the tenderloin. It was expected that this ration would show considerably better reproduction than ration 120, which also contained 15 per cent protein with added lard and cod liver oil mixed into the ration. Such was not the case, however.

Reproduction on ration 147 (kidney) was considerably better than on 137 (liver), and here again the most probable explanation is that kidney protein is superior to liver protein. Moreover, this superiority possibly appears to better advantage in the raw meats than in the cooked and dried preparations.

A comparison of the liver and kidney ratings of Series I and VII, up to the time the animals were 150 days of age, gives results as follows:

	Ration	Av. No. born per Female	Per cent which lived
Liver	130 (1st lot)	6.0	50.0
	130 (2nd lot)	9.3	10.7
	137	4.1	30.3
Kidney	140	2.3	0.0
	147	5.8	19.2

It is difficult to explain why reproduction on ration 137 was not so good as on ration 130. It can be said, however, that considering the first and second generation animals throughout life, ration 137 was better because fertility lasted longer. The second generation male on ration 137 had normal testes weight, while the second generation males on ration 130 all showed degeneration.

Ration 147 was considerably better than 140, both for reproduction and lactation. The difference in lactation is no doubt partly due to the fact that in ration 140 there had been some destruction of vitamin B in the preparation of the dried kidney. The fact that the second generation animals on ration 147 were fertile is another point in favor of this ration. This was probably the result of omitting the lard and cod liver oil from the main bulk of the food.

## SUMMARY AND CONCLUSIONS

In this comparative study, beef round, liver and kidney, in both cooked and raw forms, were fed to rats in order to determine the relative efficiency

of these foods as sources of protein and accessories, primarily for reproduction and lactation. The rations contained sufficient amounts of the meats being tested to supply 15 and 20 per cent protein. Various supplements were used, depending on the particular food constituent being tested for.

Variations in the proportions of protein and fat in the ration involved changes in the amount of lard used as a source of added fat. Rations in which lard was omitted entirely, proved better for reproduction than those which contained even a small amount. Cod liver oil was also found to have a detrimental effect on reproduction when mixed into large quantities of the rations. Therefore, in all of the later rations this oil was either added daily to small amounts of the rations or omitted entirely and partly replaced by radiation.

When no supplements of vitamin E were made, the meats themselves being relied upon as a source of this vitamin, the rations, containing only 15 per cent of protein with lard and cod liver oil, thus contained two variable factors since these added fats had a destructive effect on the vitamin. This made the interpretation of results as to the relative value of the proteins, as such, rather difficult. When, however, vitamin E was supplied in adequate amounts, the superiority of kidney protein seemed to be clearly indicated. Liver protein ranked next, and muscle last.

When the difference in the quality of the proteins was largely ruled out by feeding them at a 20 per cent level, the vitamin E content of the dried meats was found to decrease in the following order: Round, liver, kidney.

It seems probable that 5 per cent of yeast does not provide enough vitamin B (or B<sub>1</sub>) for highly successful lactation in those rations containing dried meats. This was indicated by the fact that lactation on ration 120 (muscle) was distinctly improved by addition of the alcoholic extract of ether-extracted wheat embryo, which is rich in vitamin B (or B<sub>1</sub>). Additional yeast, together with wheat germ oil, also caused improvement in lactation on rations 136 (liver) and 146 (kidney). The superiority of liver over the other meats for this function was no doubt due to its content of vitamin B, particularly the heat-labile fraction.

It is thus difficult to say which of the three meats is best for reproduction and lactation, unless it is specified in what form and amount the food is to be fed, and with what supplements. If fed in the dried form at a 20 per cent protein level in a ration containing no substances which would

have a destructive effect on vitamin E, beef round probably requires supplements of minerals and vitamins A, B, and D. Beef liver and kidney probably require in addition a supplement of vitamin E. In the raw state beef round is not easily assimilated by rats, but liver and kidney appear to have a higher food value when raw than when cooked.

It is not known whether cooking has any detrimental effect on the proteins of meat, but a large part of the vitamin B (antineuritic) must be destroyed. Of the raw meats, kidney would seem to require the fewest supplements for successful reproduction and lactation.

The author wishes to acknowledge her indebtedness to Professor Henry A. Mattill for helpful advice throughout the work.

#### BIBLIOGRAPHY

- Anderegg, L. T., and V. E. Nelson, 1926, *Jour. Ind. and Eng. Chem.*, XVIII, 620.  
Chick, H., and M. H. Roscoe, 1927, *Biochem. Jour.*, XXI, 698.  
Daniels, A. L., and M. K. Hutton, 1925, *Jour. Biol. Chem.*, LXIII, 143.  
Evans, H. M., and K. S. Bishop, 1922, *Jour. Metabol. Research*, I, 335.  
Evans, H. M., and K. S. Bishop, 1923, *Jour. Metabol. Research*, III, 201.  
Evans, H. M., 1924, *Science*, LX, 20.  
Evans, H. M., and G. O. Burr, 1927, *Memoirs of the Univ. of California*, VIII.  
Evans, H. M., and G. O. Burr, 1928, *Jour. Biol. Chem.*, LXXVI, 263.  
Goldberger, J., G. A. Wheeler, R. D. Lillie, and L. M. Rogers, 1926, *Pub. Health Reports, U. S. P. H. S.*, XLI, 297.  
Hartwell, G. A., 1921, *Biochem. Jour.*, XV, 140.  
Hartwell, G. A., 1924, *Biochem. Jour.*, XVIII, 785.  
Hassan, A., and J. C. Drummond, 1927, *Biochem. Jour.*, XXI, 653.  
Hoagland, Ralph, and G. G. Snider, 1926, *Jour. Agri. Research*, XXXII, 1025.  
Holm, G. E., G. R. Greenback and E. F. Deysher, 1927, *Jour. Ind. and Eng. Chem.*, XIX, 156.  
Mattill, H. A., J. S. Carman, and M. M. Clayton, 1924, *Jour. Biol. Chem.*, LXI, 729.  
Mattill, H. A., 1927, *Jour. Amer. Med. Assoc.*, LXXXIX, 1505.  
McCollum, E. V., and M. Davis, 1915, *Jour. Biol. Chem.*, XXI, 615.  
McCollum, E. V., N. Simmonds, and H. T. Parsons, 1921, *Jour. Biol. Chem.*, XLVII, 111.  
Mitchell, H. H., and G. G. Carman, 1926, *Jour. Biol. Chem.*, LXVIII, 183.  
Mitchell, H. H., and J. R. Beadles, 1926-1927, *Jour. Biol. Chem.*, LXXI, 429.  
Mitchell, H. H., J. R. Beadles, and J. H. Kruger, 1927, *Jour. Biol. Chem.*, LXXIII, 767.  
Osborne, T. B., and L. B. Mendel, 1917, *Jour. Biol. Chem.*, XXXII, 309.  
Osborne, T. B., and L. B. Mendel, 1918, *Jour. Biol. Chem.*, XXXIV, 17.  
Sherman, H. C., and F. L. MacLeod, 1925, *Jour. Amer. Chem. Soc.*, XLVII, 1658.  
Simmonds, N., 1924, *Amer. Jour. Hygiene*, Sept. sup. IV.  
Simmonds, N., J. E. Becker, and E. V. McCollum, 1927, *Jour. Amer. Med. Assoc.*, LXXXVIII, 1047.  
Smith, M. I., and E. G. Hendrick, 1926, *Pub. Health Reports, U. S. P. H. S.*, XLI, 201.  
Steenbock, H., and E. M. Nelson, 1923, *Jour. Biol. Chem.*, LVI, 355.  
Sure, B., 1926, *Jour. Biol. Chem.*, LXIX, 53.  
Sure, B., 1927, *Jour. Biol. Chem.*, LXXIV, 55.  
Sure, B., 1928, *Jour. Biol. Chem.*, LXXVI, 685.  
Watson, B. P., 1907, *Brit. Med. Jour.*, I, 193.  
Watson, C., 1906, *Jour. Physiol.*, XXXIV, 111.





# THE EFFECT OF VARYING AMOUNTS OF MEN- HADEN OIL IN THE DIET ON THE COM- POSITION OF THE BODY FAT OF THE WHITE RAT. THE STORAGE OF HIGHLY UNSATURATED FATTY ACIDS

By

J. B. BROWN AND S. G. MORRIS

*(From the Laboratory of Physiological Chemistry, College of Medicine, Ohio  
State University, Columbus)*

Received for Publication—October 29, 1929

THE influence of the fat of the diet on the composition of mammalian body fat is now generally recognized. Excellent reviews of this subject are given by Leathes (1) and by Lewkowitsch (2). While in general the fat from animals of the same species is fairly typical, this is due more to the fact that individuals of the same species live under similar conditions of diet and environment. Changes in these, particularly in the diet, may profoundly alter the composition of the body fat. The earlier work on this subject will not be reviewed here. However, several investigations pertaining thereto have appeared recently. Ellis and Isbell (3), in a study of the soft pork problem, produced lards with iodine numbers of 52 to 97 by the use of controlled experimental diets. The feeding of soy bean and peanut oils produced especially unsaturated fats. Anderson and Mendel (4), in a detailed investigation of the effect of diet on the body fat of white rats, obtained specimens of fat with iodine numbers from 55 to 93. They found, in agreement with earlier investigators, that previous depletion of the store of body fat by starvation resulted in greater changes. Eckstein (5) studied the effect of five diets on the composition of the lipids of the white rat and obtained lipids with iodine numbers from 47 to 88. Myristic and oleic acids from the diet were deposited while butyric acid was not.

In a previous report from this laboratory (6) it was shown that when a diet containing 20 per cent whale oil was fed to the white rat, equilibrium was established between the diet and the depot fat in from four to six weeks. The presence of increased amounts of highly unsaturated fatty acids in the stored fat was demonstrated by means of their ether-insoluble bromine addition products. Unfortunately, the whale oil used gave bromides which contained very close to the theoretical amount of bromine

for octobromoarachidic acid (67.78 per cent) so that it was uncertain whether the acid deposited was arachidonic acid, or a mixture of the whale oil acids. Usually the fish oil fatty acids yield bromides which contain about 2 per cent more bromine (69–70 per cent) than octobromoarachidic acid. Hence they may be distinguished readily from arachidonic acid. Since they apparently are deposited without difficulty in mammalian fat and are detected easily, fish oils may be used in studying certain phases of fat metabolism.

The amount of arachidonic acid present normally in the body fat of the white rat is very small (5), not over a fraction of a per cent. In several instances in this laboratory, specimens of normal rat fatty acids have yielded no ether-insoluble bromides, thus showing the absence of this acid.

In the present work, the effect of varying amounts of menhaden oil in the diet of white rats on the composition of their fat has been followed. Menhaden oil contains acids of the  $C_{16}$ ,  $C_{18}$ ,  $C_{20}$  and  $C_{22}$  series with from three to six double bonds (7). The oil used in this experiment was purchased on the market as refined menhaden oil and had an iodine number of 169.7. Its fatty acids gave 54.8 per cent of their weight of ether-insoluble bromides, containing 69.14 per cent bromine. When the rats were fed diets containing 0 to 30 per cent of this oil, increasing amounts of highly unsaturated acids were deposited in the body fat, the results showing 1.3 per cent in the controls on a diet containing 10 per cent olive oil, to 17.2 per cent in the rats on 30 per cent menhaden oil diet. In every instance the bromides isolated from the depot fatty acids contained more bromine than those from the acids of the original oil, the excess being sufficient to suggest either that the more unsaturated fatty acids of higher molecular weight were selectively deposited or that desaturation took place in the body.

#### EXPERIMENTAL PART

*Description of Experiment.* Six groups of seven adult rats each were partially starved over a period of four weeks by feeding insufficient amounts of the following control mixture,—

Whole wheat flour	653 gms.
Casein	160 "
Milk powder	160 "
NaCl	11 "
Chalk, precipitated	16 "

To each kilogram of dry mixture was added 4 cc. of cod liver oil and sufficient water to make a workable dough. The dough was spread in thin

layers and partially dried in air. The small amount of cod liver oil, added for its vitamin content to the basal mixture, was believed to be insignificant so far as its effect on the body fat was concerned. In the light of the results shown by these experiments, however, this oil may account for part at least of the 1.3 per cent unsaturated acids found in the depot fat of the control animals. At the end of the period of partial starvation, the six groups were placed on the diets described in Table I. The diets

TABLE I

Diet Per cent	Wt. dry mixture	Wt. Oil	Wt. Water	Per cent total calories as fat
	grams	grams	grams	
control	1000	111 olive oil	400	22
5	1000	52.6 menhaden oil	450	12
10	1000	111 " "	400	22
15	1000	176.4 " "	400	31
20	1000	250 " "	350	39
30	1000	428.6 " "	300	52

were kept cold and protected from oxidation as much as possible. To be more certain that oxidation did not occur, during the latter part of the experiment each diet was prepared every other day in sufficient quantities to be consumed relatively fresh. A record was kept, so far as possible, of the amount eaten by each group of rats during six weeks of feeding, these data being given in Table II.

TABLE II  
FOOD AND OIL CONSUMPTION

		5%	10%	15%	20%	30%
Total weight eaten, gms.	3942	3692	4098	3970	3093	2458
Weight eaten on dry basis, gms.	2838	2547	2950	2858	2381	1969
Weight of menhaden oil consumed	283	127	295	428	476	590
(Control olive oil)						

The weight changes during the course of the experiment are given in Table III.

The physical condition of all of the rats at the end of the experiment was good. No evidence of any deleterious effects was observed even in the 30 per cent diet, in which over half of the total calories ingested were derived from menhaden oil. During the period no general increase in weight was observed.



TABLE III  
AVERAGE CHANGES IN WEIGHT DURING EXPERIMENT

Average weight	Control	5%	10%	15%	20%	30%
At beginning	224	220	228	237	232	229
After four weeks partial starvation	197	176	186	198	205	201
After one week on diet	230	203	221	221	230	207
After six weeks on diet	231	209	238	229	237	225

#### RESULTS OF FEEDING MENHADEN OIL

At the end of the period of experimental feeding, five rats from each group were killed with chloroform, skinned, and the body fat, including that in the abdominal cavity, was removed and rendered by heating in a small flask at 130° for an hour under reduced pressure. The fat was taken up in ether, and the residue ground up and extracted with ether. The ether was removed by warming under reduced pressure. The product was kept in a small bottle under carbon dioxide. The oils were pale amber colored. On standing at room temperature, stearin settled out, the largest amount forming in the 5 per cent diet.

The oils were analyzed by the usual methods. For iodine number the Hanus method with one-half hour reaction was employed. The polybromide number was determined by brominating a 2 to 3 gram sample of oil or fatty acids in a weighed 50 cc. centrifuge tube in cold dry ether. An excess of bromine was added slowly, and the reaction allowed to stand at least four hours. The ether was removed by centrifugation, more ether was added and the bromides thoroughly stirred, and the tube again centrifugated. This washing was repeated four times, and the final product, consisting of pure white bromides, was dried at 50° and weighed. Bromine was estimated by the Parr peroxide bomb method. The per cent of highly unsaturated fatty acids found in the fatty acids was calculated by a method described previously for arachidonic acid (8). The polybromide number of the pure highly unsaturated acids of menhaden oil has been found to be 105 (7). A polybromide number of 10.19 would therefore represent  $10.19 \times 100 / 105$  or 9.7 per cent of highly unsaturated fatty acids. This method is admitted to be only approximate but the writer believes it to be more accurate than merely to calculate the acid content of the bromides, which gives a result far too low.

Results of the analyses of the oils and the corresponding fatty acids obtained from them are given in Table IV.

TABLE IV  
CHARACTERISTICS OF BODY OILS

Diet Per cent of fat	Fats				Fatty Acids			
	Sapon. number	Iodine number	Poly- brom. number	Refrac. index	Iodine number	Poly- brom. number	% Br in bromides	% HUFA*
Control	197.3	73.16	.56	1.4648	82.59	1.42		1.3
5	199.3	82.71	5.89	1.4663	90.39	10.19	70.09	9.7
10	197.3	94.67	14.95	1.4691	98.05	15.16	69.58	14.4
15	194.4	99.01	15.73	1.4697	99.60	15.63	69.87	14.9
20	196.0	97.24	18.81	1.4671	104.7	16.94	69.65	16.1
30	196.0	103.35	18.80	1.4710	104.0	18.08	70.02	17.2
Menhaden oil	191.9	169.7		1.4828	175.9	54.8	69.14	52.2

\* Highly Unsaturated Fatty Acids calculated from polybromide number.

### DISCUSSION OF RESULTS

Examination of the analytical results in Table IV shows conclusively that addition of menhaden oil to the diet of the white rat results in deposition of the characteristic highly unsaturated fish oil fatty acids in quantities roughly proportional to the amount of oil in the diet. The changes in saponification number were not so great as might be expected, since this constant for menhaden oil is appreciably less than it is for rat fat. However, the iodine number of the fat varied from 73 in the control with 10 per cent olive oil in the diet to 103 with 30 per cent menhaden oil. The iodine number of the fatty acids rose from 82 to 104 under the same conditions. The polybromide number of the control fatty acids was 1.42. If calculated as arachidonic acid, this would represent 1.8 per cent of this acid. (The polybromide number for pure arachidonic acid is 80.) If calculated as the highly unsaturated acids of menhaden oil, the content of these would be 1.3 per cent. The polybromide number rose rapidly with increase of fish oil in the diet to 18.08, representing 17.2 per cent of highly unsaturated acids.

The data in Table V compare the per cent highly unsaturated acids in the body fat with that of the diet (calculated). It is interesting to note that with the 5 per cent diet the ratio of the former to the latter is about 4:1. This ratio decreased rapidly to about 1:1 with the 30 per cent diet. In other words, there appeared to be a concentration of unsaturated acids in the body fat which was more pronounced in the lower diets. The amount

TABLE V  
A COMPARISON OF THE CONTENT OF HIGHLY UNSATURATED FATTY ACIDS OF THE BODY FAT  
AND OF THE DIET

Diet Per cent of Oil	Per cent highly unsaturated fatty acids		
	In Body Fat	In Diet	Ratio
Control	1.3	0.0	—
5	9.7	2.6	4:1
10	14.4	5.2	3:1
15	14.9	7.8	2:1
20	16.1	10.4	1.5:1
30	17.2	15.7	1:1

of highly unsaturated acids deposited became nearly constant in the 20 and 30 per cent diets.

From a theoretical standpoint, the results of analysis of the bromides from the body fatty acids are particularly interesting. In every instance they contained from 0.50 to 1.0 per cent more bromine than the bromides obtained from the original menhaden oil fatty acids. The deposited highly unsaturated acids were therefore more unsaturated than those in the fish oil. The theoretical iodine number of the acids in a bromide containing 69 per cent bromine is 352, and for a bromide containing 70 per cent bromine, 370. It is known that the highly unsaturated acids of menhaden oil contain acids from the  $C_{16}$  series with three double bonds (hexadecatrienoic) to  $C_{22}$  with six double bonds (docosahexenoic) (7). When these acids are fractionated, the bromine content of the bromides of the fractions increases with boiling point. Two explanations may be offered, therefore, to account for the increase in unsaturation. The rat may metabolize the  $C_{16}$  and  $C_{18}$  acids of the fish oil more readily than the  $C_{20}$  and the  $C_{22}$  acids, thus removing those of lower molecular weight and of lesser unsaturation and depositing the higher molecular weight, more unsaturated acids. The second possibility is suggested by the Leathes desaturation theory. The highly unsaturated acids may be still further desaturated in the body with the introduction of more double bonds. The resultant acids would give the constants found.

With such small quantities of material, and with only the bromine analyses with which to work, it is of course impossible to settle the question as to the nature of the changes taking place in the acids. It is our hope to get further light on the question by feeding larger animals, such as the hog, where large enough samples of depot fat may be obtained to make it possible actually to isolate the deposited highly unsaturated acid for comparison with those obtained from the fish oil that is fed.

## SUMMARY

1 The characteristic highly unsaturated fatty acids of menhaden oil are deposited in the body fat of the white rat when these acids are included in the diet.

2 Diets containing 5 to 30 per cent of menhaden oil resulted in a deposition of highly unsaturated acids from 9.7 to 17.2 per cent.

3 In the diets studied, the content of highly unsaturated acids of the depot fat was always higher than that of the diet.

4 The highly unsaturated acids deposited were more unsaturated than those from the original menhaden oil.

## BIBLIOGRAPHY

1. Leathes, J. B., *The Fats*. Second Ed. 1925, 96.
2. Lewkowitsch, J., *Chemical Technology and Analysis of Oils, Fats, and Waxes*, II, Sixth Ed. 679.
3. Ellis, N. R., and Isbell, H. S., *Jour. Biol. Chem.*, 1926, LXIX, 219-239.
4. Anderson, W. E., and Mendel, L. B., *Jour. Biol. Chem.*, 1928, LXXVI, 729.
5. Eckstein, H. C., *Jour. Biol. Chem.*, 1929, LXXXI, 613.
6. Brown, J. B., and Rawlins, A. L., *Proc. Soc. Exp. Biol. Med.*, 1929, XXVI, 704.
7. Brown, J. B., and Beal, G. D., *Jour. Amer. Chem. Soc.*, 1923, XLV, 1289.
8. Brown, J. B., *Jour. Biol. Chem.*, 1929, LXXXIII, 777.



---

## Editorial Review<sup>1</sup>

### THE CHEMISTRY OF VITAMIN D<sup>2</sup>

**I**N THE attempts which have been made to identify the vitamins, there has been found in irradiated ergosterol, a substance or mixture of substances, which can be more nearly identified with vitamin D than can any other substance with any other vitamin. Vitamin D is concerned specifically with the utilization of calcium and phosphorus in the body and its absence from the diet leads frequently to the condition known as rickets.

It was first shown experimentally in 1922 by McCollum and coworkers (1) that such a vitamin exists. By aerating cod liver oil, these investigators found that vitamin A which was then known to be present in the oil, was destroyed while another substance was left which played "an important rôle in bone growth". Soon after this, it was found that the non-saponifiable portion of the cod liver oil contained the vitamin (2).

It had been shown by Huldshinsky (3) in 1919 that the ultra-violet rays of the sun were effective in the treatment of rickets and in 1924 Hess (4) and Steenbock (5) almost simultaneously demonstrated that the feeding of a food which had been exposed to ultra-violet light was as effective as irradiating the animal itself. These investigators showed that most natural foods could be endowed with antirachitic value by irradiation with ultra-violet light. If vegetable oils were irradiated and made active antirachitically, their unsaponifiable fractions possessed the antirachitic properties just as in the case of cod liver oil. Old oils could not be activated by irradiation with ultra-violet light nor was it possible to activate their unsaponifiable fractions.

Since phytosterol is the main constituent of the non-saponifiable portion of vegetable oils, and cholesterol an important part of the non-saponifiable portion of cod liver oil, these two substances were next studied in many laboratories. When carefully purified by repeated crystallization, both were made active antirachitically on irradiation with ultra-violet rays shorter than  $315\text{ m}\mu$  (6, 7).

Hess and coworkers (8) next showed that the cholesterol which had been irradiated could be separated into an active and an inactive fraction, by

---

<sup>1</sup> As stated in the first issue of this journal, the term Editorial Review signifies not that the writer necessarily is an editor, but that the review has been read and criticized by at least four members of the Editorial Board.

<sup>2</sup> The author, Dr. Florence L. MacLeod, is Research Assistant, Department of Chemistry, Columbia University.

precipitation with digitonin, the activity being present in the non-precipitable fraction. Likewise from cod liver oil, on precipitating the cholesterol from the non-saponifiable fraction, a more active fraction was obtained. Koch and coworkers (9) and Hess and coworkers (8) both found that if cholesterol or the non-saponifiable fraction of cod liver oil were extracted with liquid ammonia, the soluble fraction had increased activity.

The different investigators now began to feel that the activity of the cholesterol was probably due to an impurity rather than to cholesterol itself. Windaus and Hess (10) pointed out that a very small part of the cholesterol underwent the change for the melting-point and the composition of the irradiated product were unchanged. They stated that the only difference in the product besides its antirachitic potency was a decrease in the absorption of ultra-violet light between 280 and 300  $m\mu$  on irradiation. While they were working Rosenheim wrote to Windaus saying that he was convinced that cholesterol contained a small amount of impurity. Pohl and Heilbron, Kamm and Morton came to the same conclusion at approximately the same time.

Rosenheim and Webster (11) showed that after irradiation they could recover at least 99.9 per cent cholesterol by precipitation with digitonin. The active substance was left unprecipitated. They also showed that an isomer of cholesterol, allocholesterol, could not be activated and further that if cholesterol were converted into the dibromide and reduced again to cholesterol, the cholesterol obtained could not be activated. So they concluded that the precursor of vitamin D was not cholesterol but a substance associated with it and one which followed cholesterol in all its stages of purification by the usual methods (esterification, saponification and recrystallization).

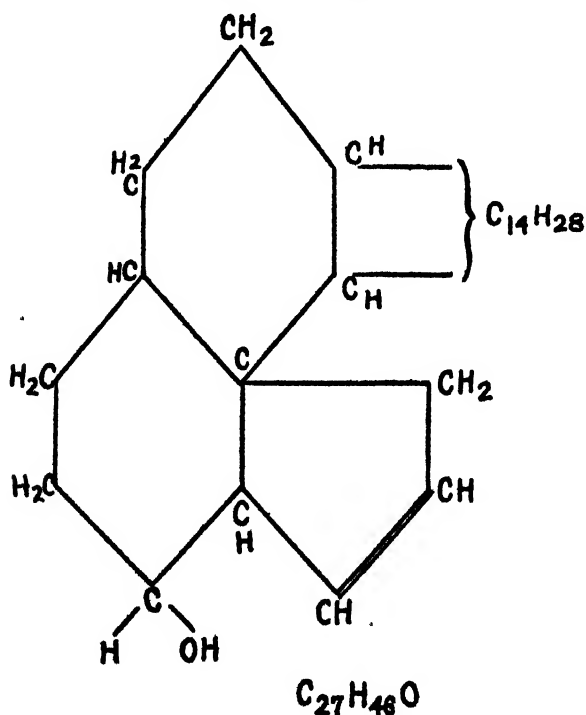
Heilbron, Kamm and Morton (12) were able by recrystallization of cholesterol from ethyl acetate to concentrate the active substance in the least soluble fraction and to show that this fraction had more clearly defined absorption bands than the original cholesterol. They felt that this was strong evidence that the substance showing the absorption bands in the ultra-violet region of the spectrum was different from cholesterol and was probably the precursor of the vitamin. Windaus and Hess (10) were able to show similarly that cholesterol purified by way of the dibromide did not exhibit antirachitic activity on irradiation with ultra-violet rays and did not exhibit the characteristic absorption bands. They also freed the cholesterol from the "provitamin", as they called the impurity, by prolonged boiling with blood charcoal, and by treatment with potassium

permanganate, and were able to concentrate it, in the least soluble fraction, by recrystallization from acetic acid, and by high vacuum distillation.

They found that the "provitamin" was precipitated by digitonin before irradiation and that it must be an unsaturated compound for if it were hydrogenated, it no longer could be activated by ultra-violet light.

The sensitiveness of the "provitamin" to oxidative processes is characteristic of one of the known sterols, ergosterol. Tanret (13) who described ergosterol in 1908 had studied its behavior under the influence of light and air as evidenced by change in color, fall of melting point and change in optical activity. The behavior of the "provitamin" suggested to Windaus that it possibly was ergosterol, which is similar in structure to cholesterol but has three double bonds instead of one and like cholesterol has one OH group.

The latest formula which Windaus (10) gives for cholesterol is:



All that is known of ergosterol is that there are two more double bonds in its molecule but it is not known where they are. Its empirical formula then is C<sub>27</sub>H<sub>44</sub>O. Tanret originally prepared it from the fungus, ergot, but at present it is commonly obtained from yeast.

Ergosterol, one part in 600,000 parts of absolute alcohol, was found by



Windaus and Hess to give the same absorption spectrum as a one per cent solution of cholesterol. A pure cholesterol, free from the "provitamin", was mixed with 1/60 per cent of ergosterol and a product was obtained which was not separable from cholesterol by crystallization, melting-point, optical activity or composition. The irradiated ergosterol was much more potent antirachitically than cholesterol.

Pohl (14), working with the same materials as Windaus, showed also that the absorption spectra of cholesterol and ergosterol were similar. Pohl found that after irradiation for 25 minutes the ergosterol showed an absorption band only at about  $240\text{ m}\mu$ , instead of exhibiting maxima at 293, 280 and  $270\text{ m}\mu$ , and attributed this band to vitamin D. Morton, Heilbron and Kamm (15) studied at half-hour intervals the changes in the absorption spectrum brought about by the irradiation of ergosterol in alcohol solution. After two and a half hours they obtained an absorption band at  $247\text{ m}\mu$  which they also attributed to vitamin D with only incidental tests to prove the antirachitic value of the product.

Bills, Honeywell and McNair (16) by means of the spectroscope showed roughly the percentage of ergosterol in some cholesterol which they had obtained from spinal cord by extraction with acetone, saponification in alcoholic potassium hydroxide and crystallization from alcohol. The cholesterol gave faint absorption bands at 293, 282, 270 and  $260\text{ m}\mu$ . A 0.003 per cent solution of ergosterol gave less absorption than a three per cent solution of cholesterol and a 0.004 per cent solution somewhat more. From this they concluded that the cholesterol with which they were working contained  $1.2 \pm 0.1$  parts per 1000 of ergosterol.

They then reported experiments contradicting in some respects the reports of Windaus and Hess and of Rosenheim and Webster. By prolonged treatment of cholesterol either with potassium permanganate or with charcoal or by bromination and reduction back to cholesterol, Bills and coworkers were unable to completely destroy the activatability of the cholesterol. By their technique they were able to detect a smaller degree of activation than the other workers. In addition to giving positive biological tests, Bills was able to show that the purified cholesterol gave five faint absorption bands at 315, 304, 293.5, 282 and  $269\text{ m}\mu$ . From these results he and his coworkers were unable to decide whether the activation of the cholesterol was entirely due to ergosterol. The absorption bands at 293.5, 282 and  $269\text{ m}\mu$  would indicate that it was, whereas they felt that it was difficult to believe that bromination which destroyed more than 99 per cent of ergosterol when applied once, should fail to destroy the remainder when applied a second time. Jendrassik and Keményfi (17)

reported at about the same time that cholesterol could still be activated after purification by way of the dibromide and they believed that ergosterol was not the only substance capable of being activated.

Koch, Koch and Lemon (18) have recently published some experiments confirming these results of Bills. They found that on purifying cholesterol by the bromine treatment or by a triple treatment with potassium permanganate, the two products upon irradiation induced calcification in dosages 70 to 30 times as large as that required of the original cholesterol. The two products showed no absorption bands corresponding to ergosterol and showed no general absorption in the ultra-violet region.

These investigators do not believe this low activatability to be due to a trace of ergosterol. When their purified products were heated slightly above the melting point under conditions that avoided appreciable oxidation, every one of the purified products was practically 25 to 70 times as active as the presumably pure cholesterol. With the increase in activatability a strong general absorption was observed in the ultra-violet region but since there were no bands whatever they feel that the potency of the product is not due to ergosterol. They believe that "provitamin D activity" may be a general property in varying degrees of various sterols or of certain forms of those sterols.

In spite of these rather disturbing findings, most of the work which has been done recently on this subject has been carried out on ergosterol, to see what changes take place in the absorption spectra under different conditions of irradiation, and what happens to the ergosterol molecule on irradiation.

Spectroscopic studies on cholesterol and later on ergosterol have been made for the most part by Bills in this country, by Rosenheim, Webster and Bourdillon and by Heilbron, Morton and Kamm in England and by Pohl in Germany.

It was shown by Pohl (14) and Morton, Heilbron and Kamm (15) that when ergosterol is irradiated for some time the three absorption bands at 293.5, 282 and 270  $m\mu$  disappear while a new band appears at 247  $m\mu$ . As was pointed out earlier in this paper, these investigators assumed without adequate biochemical control that the substance giving the absorption band at 247  $m\mu$  was vitamin D.

Bills and coworkers (19) undertook similar experiments in which they studied the effect of irradiation with ultra-violet light on the absorption spectrum of ergosterol over a long period of time, conducting parallel spectroscopic and feeding tests. The ergosterol was of exceptional purity giving an  $[\alpha]_D^{20} = -132^\circ$  in chloroform which would indicate a high

degree of purity, one gram being dissolved in a liter of optically pure 95 per cent alcohol. The solution was activated in a homogeneous quartz absorption cell, 2 cm. deep, completely filled. A water-cooled mercury lamp was used and the light was reasonably constant with uniform voltage and temperature. The absorption curves were made with a Hilger quartz spectrograph. Fresh cellfules were used for irradiating the solutions for each period of exposure. A portion of each irradiated alcoholic ergosterol solution was used for photographing the absorption spectra while the rest was used for the biological tests.

The results of this investigation were as follows:—

*Non-irradiated stock solution.* This solution gave absorption bands with maxima at 270 and 282  $m\mu$ . The band at 293.5  $m\mu$  was evident, a fourth band at 260  $m\mu$  which had been detected by Bills and MacNair (16) in previous experiments was too faint to be recognized. The non-irradiated solution had no antirachitic activity.

*Irradiated 7-1/2 minutes.* The absorption curve resembled that of the non-irradiated solution with the maxima a little lower. There was no evidence of any new absorption band yet the tests revealed the development of antirachitic activity 150,000 times as great as that of cod liver oil.

*Irradiated 15 minutes.* There was a slight change in the absorption curve but no evidence of a new band. The activation nearly reached its maximum at this point, the potency being 225,000 times that of cod liver oil.

*Irradiated 22-1/2 minutes.* The absorption band at 270  $m\mu$  broadened but there was little change in the absorption curve. With this length of time of irradiation the activation reached its highest point with a potency 250,000 times that of cod liver oil.

*Irradiated 30 minutes.* The maximum at 293.5  $m\mu$  disappeared, that at 282  $m\mu$  was still conspicuous and that at 270  $m\mu$  showed a distortion which indicated the development of a new region of absorption in the shorter wave-lengths. The potency declined to 200,000 times that of cod liver oil.

*Irradiated 1 hour.* The maximum at 282  $m\mu$  nearly disappeared, that at 270  $m\mu$  was barely evident and the new maximum began to become distinct at about 252  $m\mu$ . The potency continued to decline.

*Irradiated 2 hours.* All the absorption maxima of ergosterol disappeared. The new band became more intense with its maximum at about 250  $m\mu$ . The antirachitic potency declined to 50,000 times that of cod liver oil.

*Irradiated 3 hours.* Coincident with the development of the new band at 248  $m\mu$  in its greatest intensity, the antirachitic potency almost disappeared.

*Irradiated 4, 5, 7-1/2, 10 and 15 hours.* Between 3 and 4 hours, the absorption band at 248  $m\mu$  faded slightly; during 4 to 7-1/2 hours no further change was observed; after 7-1/2 hours the fading resumed. The antirachitic potency never reappeared.

From this it is evident that the photochemical reaction product which exhibits the absorption band at 248  $m\mu$  is not vitamin D. The appearance of this band coincides not with the development but rather with the destruction of antirachitic potency. Bills says that it is a by-product of the activation of ergosterol or a degradation product of the vitamin.

The activation curve considered with the absorption curve shows that the antirachitic potency is developed rapidly reaching a maximum when

about 73 per cent of ergosterol is still present. The decline in potency commences at the point where on account of a depleted reserve of ergosterol and consequent decline in the rate of formation of the vitamin, the decomposition overtakes the formation.

When one cellful of solution was used and samples were withdrawn from time to time, leaving more and more air in contact with the residual liquid, the band at  $248\text{ m}\mu$  developed and faded much more rapidly. The amount of oxygen in the air contained in the partly filled cell was sufficient for considerable oxidation. The oxidation causes a destruction in the  $248\text{ m}\mu$  band and does not affect the formation of vitamin D.

The substance showing the absorption band at  $248\text{ m}\mu$  is clearly related to ergosterol and vitamin D. Bills points out that isoergosterol, described by Reindel, Walter and Rauch (20) exhibits just such a band. The isoergosterol was obtained by the addition and then the removal of hydrochloric acid from the ergosterol molecule. The absorption of isoergosterol is from  $220\text{ m}\mu$  to  $270\text{ m}\mu$  with a maximum at  $248\text{ m}\mu$ . This band fades also on prolonged irradiation. It is not at all certain that isoergosterol and the substance formed by the irradiation of ergosterol are identical but Bills feels that with the formation of vitamin D by light a substance is formed which must have a molecular configuration not unlike isoergosterol. This same suggestion, that the absorption band at  $248\text{ m}\mu$  is due to isoergosterol, has been made by two other investigators, van Wijk and Reerink (25).

Of interest in connection with this paper by Bills is a report of Rosenheim and Webster (21) in which they showed, by precipitation with digitonin, that after a half hour of irradiation, 90 per cent of unchanged ergosterol is left whereas after 4 hours there is 10 per cent of ergosterol and the antirachitic potency is the same. The conditions of their experiments were probably different and not so carefully controlled as those of Bills but the results do indicate that the activity of the reaction products do not continue to increase with the disappearance of ergosterol.

Bourdillon, Webster and coworkers (22) hold the theory that the ultra-violet irradiation of ergosterol produces three substances in succession. The first, which they call A, shows intense absorption for wavelengths of  $250$  to  $310\text{ m}\mu$  with a maximum absorption at  $280\text{ m}\mu$ . The second, called B, shows intense absorption at  $240\text{ m}\mu$  and no antirachitic power. The final product shows little or no absorption or antirachitic power. They found, unlike Bills, that the absorption curve of the substance A was greater than that of ergosterol itself. They believe this may be due to the fact that they measured their solutions sooner and also to

a lack of stirring of the solutions by Bills and others. Substance A was formed by irradiating a 0.1 per cent solution of ergosterol in absolute alcohol. 30 seconds to 10 minutes, removing the unchanged ergosterol by digitonin, evaporating and extracting with ether. The reasons for their belief that substance A is a single substance are as follows:

1. On repetition they always obtained similar spectra, whether in alcohol or in hexane, when the radiation was continued one to 10 minutes. When it was radiated to form substance B, there was a change in absorption.

2. Ergosterol and substance B were most likely to be contaminants of substance A. They believed that the absence of substance B was shown by the small absorption at  $240\text{ m}\mu$ . The absence of ergosterol was more difficult to prove. From the solubility in alcohol, they judged there was little if any ergosterol present whereas when some of the solutions were subjected to further irradiation, an increase in absorption at wave-lengths  $274$  and  $311\text{ m}\mu$  was shown, thus indicating there was, perhaps, some ergosterol present. Having given this evidence to show that A is a single substance they go on to present the following evidence for believing that substance A is vitamin D.

1. In a long series of quantitative comparisons of absorption spectra and antirachitic activity in solutions of substance A, they found a roughly linear relation between antirachitic activity and intensity of absorption at wave-lengths from  $310$  to  $270\text{ m}\mu$ . With high activity, there was high absorption and with decreased activity, lower absorption.

2. A 0.01 per cent solution of substance A in a silica cell with nitrogen stirring was exposed to ultra-violet light in which the wave lengths shorter than  $265\text{ m}\mu$  were excluded by a "light filter" of alcoholic cobalt chloride. A rapid destruction of substance A occurred by conversion of it to substance B, B having little absorption for wave-lengths longer than  $250\text{ m}\mu$  was partly protected by the filter and produced in considerable concentration. It showed high absorption at  $240\text{ m}\mu$  and no antirachitic activity. This proves that substance B is not vitamin D.

3. A solution of ergosterol was irradiated for various lengths of time and divided into two portions. In one portion the activity and the absorption were measured without previous removal of ergosterol. In the other, the per cent of ergosterol was estimated gravimetrically by precipitation with digitonin. From this they calculated the absorption of the products of radiation. The antirachitic activity was found to be proportional to the amount of substance A present. Under their conditions

there is a rapid rise in activity in the first 10 minutes, slow rise after 20 minutes and a maximum at 40 minutes, after which there is a fall.

4. Attempts were made to destroy substance A by oxidation and to observe whether the rates of disappearance of absorption at 280  $m\mu$  and of antirachitic activity were equal. The effects of bubbling oxygen through a 0.2 per cent alcoholic solution at 76° C. for one hour were too small for certainty. But when the solution was evaporated to dryness, the exposure of the residue to gaseous oxygen at 100° C. produced a more rapid effect. A pale brown solid residue was formed of reduced solubility in alcohol, with reduced antirachitic activity and reduced absorption at 280  $m\mu$ . They think this shows that substance A and vitamin D offer marked resistance in alcoholic solution to oxygen at 76° C., but when dried and subjected to oxygen at 100° C. they are (or it is) oxidized with speed.

5. The fifth piece of evidence which they give is that they have never obtained a solution showing strong absorption at 280  $m\mu$ , that did not show strong antirachitic activity or a solution of high antirachitic activity without absorption at 280  $m\mu$ . Vitamin D may be a substance in substance A.

6. They give a much less direct argument as their sixth. They say that in preparations of A, the antirachitic activity is destroyed rapidly by radiations between 330 and 260  $m\mu$ . Therefore, the vitamin D absorption band lies between these. Attempts to produce it from other sterols failed. If two hydrogen atoms are added to one double bond, the substance is not activated. This makes it probable that vitamin D has the three double bonds of ergosterol.

Hence on the assumption that the preparations of substance A are a mixture of one absorbing substance with other non-absorbing products, it is probable that the absorbing substance is vitamin D. The other hypothesis is that vitamin D of enormous biochemical activity is found in small percentage in substance A. The best preparations of substance A are 2 to 4 times as strong as preparations of which the antirachitic action can be detected in doses of 1/100000 mg. per day. The activity of the hypothetical substance is very high indeed.

7. For the last argument they state that when ergosterol is irradiated the absorption increases regularly. In the earliest stages the increase is proportional to the time of radiation. This shows a direct transition from ergosterol to A. The transition from A to B is the same. It is improbable that vitamin D is an intermediate stage. They point out that the pos-

sibility of the formation of vitamin D directly from ergosterol by an independent reaction is not excluded.

They conclude from all this, that there is good reason to believe that the vitamin D absorption spectrum closely resembles that of substance A. It is possible that A is merely a by-product formed at the same time as vitamin D or that D and A are two closely similar substances related by some reversible process such as condensation or polymerization.

The preparations of substance A were colorless, glassy masses which failed to crystallize. There is the question whether the preparations contained a small quantity of a substance of high specific absorption or a large quantity of a substance of lower absorption. The question is of interest since estimates of the absolute activity of vitamin D have depended on assumptions as to quantum efficiency which they feel are of doubtful validity. They believe that the substance A which was formed by irradiating a 0.1 per cent solution of ergosterol for 30 seconds to 10 minutes, to form as little B and C as possible, and precipitating out the unchanged ergosterol with digitonin, is a single substance and probably vitamin D. From their results they calculated that the smallest amount of pure vitamin they should detect is  $1.9 \times 10^{-8}$  gram. This value is very close to the amount reported by Fosbinder, Daniels, Steenbock and Kon (23, 24) in this country but the authors feel that the results are so dependent on conditions that the fact that the calculations agree so closely is of little significance.

Fosbinder, Daniels and Steenbock (23) and Kon, Daniels and Steenbock (24) made a quantitative study of the photochemical activation of sterols and attempted to determine the amount of energy sufficient to produce, when fed, a demonstrable deposition of calcium in a rachitic animal. First working with cholesterol at the 265 m $\mu$  wave-length, they found that an energy input of 234 ergs was necessary to give positive antirachitic potency with an exposure of 22.5 seconds. Making use of the quantum theory, they calculated that  $3.2 \times 10^{13}$  quanta were involved. According to Einstein's law of photochemistry,  $3.2 \times 10^{13}$  molecules of vitamin D were synthesized by  $3.2 \times 10^{13}$  quanta during the exposure of 22.5 seconds. The number of gram molecules were then obtained by dividing the number of molecules by the Avogadro number, which gives  $5 \times 10^{-11}$  gram molecule. Then assuming that the molecular weight of the antirachitic material was essentially the same as that of cholesterol (385), the number of grams of vitamin becomes  $5 \times 10^{-11} \times 385$  which equals  $2 \times 10^{-8}$  gram. This indicates that 20 billionths of a gram or 20 millionths

of a milligram of vitamin D is sufficient to produce calcium deposition in a rachitic rat.

They feel that the assumption of the Einstein law is justified as a first approximation. The assumption is also made, of course, that all the light absorbed by purified cholesterol is converted into vitamin D. If some of the light were absorbed by material which was not converted into vitamin D, the quantity found by these calculations would be still further reduced.

The work was repeated using purified ergosterol. This time the ergosterol was irradiated at the 256, 265, 280 and 293  $m\mu$  lines and the threshold value of activation for all wave lengths was found to be 700–1000 ergs ( $10 \times 10^{18}$  to  $14 \times 10^{18}$  quanta). The lower value corresponds to  $6 \times 10^{-8}$  gram of vitamin D. The quantum efficiency of the antirachitic activation of ergosterol is smaller than that given in the earlier paper but the authors feel that these last results are more accurate.

The formation of vitamin D was thus shown to be independent of wavelength over practically the whole range of selective absorption and to be a function of the incident energy. They exposed, too, chemically purified cholesterols, purified by the severe methods which Bills believes do not wholly destroy their activatability and found that the highest amount of energy used, 200,000 ergs, was insufficient to activate them. This caused them to conclude that ergosterol is definitely the "provitamin."

It may be of interest to sum up the properties of ergosterol which are known and the changes which have been shown to take place in them upon irradiation. The melting point of ergosterol has been reported to be anywhere from  $135^{\circ}$ – $183^{\circ}$  C. (26) depending upon the purity of the sample and the degree of hydration. Upon irradiation with ultra-violet light, a glassy hard solid of indefinite melting point is obtained which begins to melt at  $30^{\circ}$  C. (30).

The  $[\alpha]_D^{20}$  in chloroform of ergosterol is  $-132^{\circ}$  according to Tanret (13), Bills (26) and others. The findings for the specific rotation seem to be in better agreement than for the melting point. When ergosterol is irradiated, its specific rotation changes gradually towards the right and after 45 hours, according to Windaus and Linsert (27) is definitely positive. However, when the  $[\alpha]_D$  becomes positive, the irradiated substance is no longer active antirachitically.

Ergosterol is slightly soluble in 95 per cent alcohol, to about 0.2 per cent, while the irradiated substance is soluble in its own weight of alcohol (30). Both are insoluble in water (30).



Windaus and Linsert (27) have shown that the molecular weight of the irradiated substance is the same as that of the unirradiated substance and that the number of double bonds is unchanged on irradiation.

Since the ergosterol itself is precipitated by digitonin and the irradiated product is not, the conclusion is that there has been some change in the OH group, probably an isomerization, since the molecular weight is the same (27).

There seems to be some disagreement as to the effect of the solvent on the potency of the irradiated ergosterol. Windaus (28), using cyclohexane, ether, normal benzene and benzol, reported that the influence of the solvent on the activity was small while Bills (29) reported in August 1929 that the solvent had a very great influence on the potency of the product of irradiation. Bills used alcohol, ether and cyclohexane. He also reported that a freely transparent solvent is not always required, for extremely high activation can be obtained in arachis oil, which is a solvent employed in commercial practice. No complete report on this work has been made by Bills.

Some investigations have been made on the irradiation of cholesterol and ergosterol at very low temperatures. Bills and Brickwedde (31) found that if cholesterol were irradiated at the temperature of liquid oxygen ( $-183^{\circ}$ ), it was about one-tenth as active as when irradiated at room temperature. Webster and Bourdillon (30) obtained similar results with ergosterol. Both concluded that the temperature coefficient of the reaction causing the formation of vitamin D is small. They argue that the reaction must be entirely photochemical and that the activation is due to an isomerization.

The irradiated substance is more stable to air and light than the unirradiated ergosterol. Irradiated ergosterol has been reported as being very active after standing one and a half years in an inactive oil (32). If oxygen is present on irradiation the absorption band at  $248\text{ m}\mu$  develops and fades more quickly than when oxygen is absent indicating that oxidation is concerned in the destruction of the substance showing the  $248\text{ m}\mu$  absorption band and not in the formation of vitamin D (19).

It seems almost unnecessary to say in conclusion that an enormous amount of work is left to be done on this subject. Successful solution of the many problems involved can come only as a result of the cooperation of the organic chemist, the physical chemist and the biological chemist.

FLORENCE L. MACLEOD

## BIBLIOGRAPHY

1. McCollum, E. V., Simmonds, N., Becker, J. E., and Shipley, P. G., 1922, Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. *Jour. Biol. Chem.*, LIII, 293.
2. Zucker, T. F., Pappenheimer, A. M., and Barnett, M., 1922, Observations on cod liver oil and rickets. *Proc. Soc. Exptl. Biol. Med.*, XIX, 167.
3. Huldshinsky, K., 1919, Heilung von Rachitis durch künstliche Höhensonne. *Deutsch. Med. Wochenschr.*, XLV, 712.
4. Hess, A. F. and Weinstock, M., 1924, Antirachitic properties imparted to inert fluids and to green vegetables by ultra-violet irradiation. *Jour. Biol. Chem.*, LXII, 301.
5. Steenbock, H. and Black, A., 1924, Fat-soluble vitamins. XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultra-violet light. *Jour. Biol. Chem.*, LXI, 405.
6. Hess, A. F., Weinstock, M., and Helman, F. D., 1925, The antirachitic value of irradiated phytosterol and cholesterol. I. *Jour. Biol. Chem.*, LXIII, 305.
7. Steenbock, H., Black, A., Nelson, E. M., Nelson, M., and Hoppert, C. A., 1925, Antirachitic activation by light. *Jour. Biol. Chem.*, LXIII, 25.
8. Hess, A. F., Weinstock, M., and Sherman, E., 1926, The antirachitic value of irradiated cholesterol. II. A separation into an active and an inactive fraction. *Jour. Biol. Chem.*, LXX, 123.
9. Koch, E. M., Cahan, M. H., and Gustavson, R. G., 1926, The antirachitic properties of certain lipoids. *Jour. Biol. Chem.*, LXVII, lii.
10. Windaus, A. and Hess, A. F., 1926, Sterine und antirachitisches Vitamin. *Nachr. Ges. Wissensch. Göttingen, Math.-physik. Klasse*, 175.  
Hess, A. F. and Windaus, A., 1926, Contaminating substances as a factor in the activation of cholesterol by irradiation. *Proc. Soc. Exptl. Biol. Med.* XXIV, 369. The development of marked activity in ergosterol following ultra-violet irradiation. *Proc. Soc. Exptl. Biol. Med.*, XXIV, 461.
11. Rosenheim, O. and Webster, T. A., 1926, Further observations on the photochemical formation of vitamin D. *Jour. Soc. Chem. Ind.*, XLV, 932.  
1927, The relation of cholesterol to vitamin D. *Biochem. Jour.*, XXI, 127.
12. Heilbron, I. M., Kamm, E. D., and Morton, R. A., 1926, The absorption spectra of cholesterol and its possible biological significance with reference to vitamin D. *Jour. Soc. Chem. Ind.*, XLV, 932.
13. Tanret, C., 1908, Sur l'ergosterine et la fongisterine. *Ann. chim. et physique*, XV, 313.
14. Pohl, R., 1926, Über das Absorptionsspektrum des antirachitischen Provitamins und Vitamins. *Nachr. Ges. Wissensch. Göttingen, Math.-physik. Klasse*, 185.
15. Morton, R. A., Heilbron, I. M., and Kamm, E. D., 1927, The absorption spectrum of ergosterol in relation to the photosynthetic formation of vitamin D. *Jour. Chem. Soc.*, 2000.
16. Bills, C. E., Honeywell, E. M., and McNair, W. A., 1928, Antirachitic substances. VII. Biochemical and spectroscopic studies on purified cholesterol. *Jour. Biol. Chem.*, LXXXVI, 251.
17. Jendrassik, A. and Keményfi, A. G., 1927, Zur Kenntnis des D-Vitasterins. I. Mitteilung: Über die Aktivierbarkeit des Cholesterins. *Biochem. Zeit.*, CLXXXIX, 180.
18. Koch, F. C., Koch, E. M., and Lemon, H. B., 1929, Fractionation studies on provitamin D. *Jour. Biol. Chem.* LXXXV, 141.  
Koch, E. M., Koch, F. C., and Lemon, H. B., 1929, Absorption spectra studies on cholesterol and ergosterol. *Jour. Biol. Chem.* LXXXV, 159.
19. Bills, C. E., Honeywell, E. M., and Cox, W. W. Jr., 1928, Antirachitic substances. IX. Quantitative biophysical studies on the activation of ergosterol. *Jour. Biol. Chem.*, LXXX, 557.
20. Reindel, F., Walter, E., and Rauch, H., 1927, Über das Ergosterin der Hefe I. *Leibig's Ann. Chem.*, 34.

21. Rosenheim, O. and Webster, T. A., 1927, The photochemical production of vitamin D from ergosterol. *Lancet*, 1927, II, 622.
22. Bourdillon, R. B., Fischmann, C., Jenkins, R. G. C., and Webster, T. A., 1929, The absorption spectrum of vitamin D. *Proc. Roy. Soc., B*, CIV, 561.
23. Fosbinder, R. J., Daniels, F., and Steenbock, H., 1928, A quantitative study of the photochemical activation of sterols in the cure of rickets. *Jour. Am. Chem. Soc.*, L, 923.
24. Kon, S. K., Daniels, F., and Steenbock, H., 1928, The quantitative study of the photochemical activation of sterols in the cure of rickets. II. *Jour. Am. Chem. Soc.*, L, 2573.
25. van Wijk, A. and Reerink, E. H., 1928, Vitamin D and iso-ergosterol. *Nature*, CXXII, 648.
26. Bills, C. E., and Honeywell, E. M., 1928, Antiricketic substances. VIII. Studies on highly purified ergosterol and its esters. *Jour. Biol. Chem.* LXXX, 15.
27. Windaus, A. and Linsert, O., 1928, Über die Ultraviolett-Bestrahlung des Dehydro-ergosterins. *Liebig's Ann.* CDLXV, 148.
28. Windaus, A., Westphal, K., v. Weider, F., and Rygh, O., 1929, Einige Beobachtungen über die Ultraviolettbestrahlung des Ergosterins. *Nachr. ges. Wissensch. Göttingen, Math.-physik. Klasse*, 45.
29. Bills, C. E., Honeywell, E. M., Cox, W. W. Jr., and Wirick, A. M., 1929, Studies on the activation of ergosterol. *Proc. XIII th Internat. Physiol. Cong. Amer. Jour.*, XC, 286.
30. Webster, T. A. and Bourdillon, R. B., 1928, Notes on the irradiation of ergosterol. *Biochem. Jour.*, XXII, 1223.
31. Bills, C. E. and Brickwedde, F. G., 1927, The activation of cholesterol at liquid oxygen temperature. *Nature*, CXXI, 452.
32. Rosenheim, O. and Webster, T. A., 1927, The parent substance of vitamin D. *Biochem. Jour.*, XXI, 389.



## INDEX

- A**BSORPTION, selective, for isolation of narrow spectral regions, 111.
- Acid control of the pylorus, new evidence on, 318.
- Aluminum content of milk boiled in aluminum, 336.
- Anemia, of young pigs, and its prevention, 277.
- , study of liquid and dry milk as anemia-producing diets, 451.
- Apparatus for collection of rat feces, 298.
- , for irradiation of rats, 126, *et seq.*
- Aubel, C. E. (See Hughes, J. S., and Lienhardt, H. F.), 183.
- Avitaminosis A, effect on nerve degeneration, 183.
- Axtmayer, J. H., A study of the vitamin B complex of red kidney beans and polished rice, 353.
- B**AKING powder residues; do they exert injurious effects upon growth and nutrition? 155.
- Beadles, Jessie R. (See Mitchell, H. H.), 225.
- Beans, red kidney, study of the vitamin B complex of, 353.
- Beef muscle, value of for reproduction and lactation, 491.
- Bing, Franklin C., and Lafayette B. Mendel, The vitamin B and the vitamin G requirements of the albino rat, 49.
- Biological values and the behavior of food and the tissue proteins, 419.
- Bischoff, Fritz (See Long, M. Louisa), 245.
- Blood counts, curves of, in rats fed on different kinds of milk, 462, *et seq.*
- Blunt, Katherine (See Whitacre, Jessie and Willard, Alice), 187.
- Body weight of the rabbit, influence of insulin upon, 245.
- Bone(s), analyses of rats irradiated through filters, 140, *et seq.*
- , analysis of rabbits on rachitic diets, method, 258.
- , breaking strength of, as affected by diet, 478.
- Bran, cause of the laxative action of, 295.
- , inorganic constituents of, 296.
- , rolled oats and, in a scurvy-producing diet and the negative control test, 171.
- Brown, J. B., and S. G. Morris, The effect of varying amounts of menhaden oil in the diet on the composition of the body fat of the white rat. The storage of highly unsaturated fatty acids, 509.
- C**ALCIUM, low, effect of on growth and metabolism of calves, 471.
- , of bones of rats on diets deficient in vitamins A and D, 262.
- Campbell, H. L. (See Sherman, H. C.), 415.
- Carbohydrate, metabolism of, after ingestion of water, 364, *et seq.*
- , protein metabolism of children on diets extremely low in, 31.
- Carman, J. S., H. G. Smith, Gladys C. Havens and J. R. Murlin, The nutritive value of cereal breakfast foods. II. Digestibility *in vitro*, with a study of methods, 91.
- Carpenter, Thorne M., and Edward L. Fox, The gaseous exchange of the human subject. I. As affected by the ingestion of water at 37.0°C., 359.
- , —, II. As affected by small quantities of dextrose, 375.
- , —, III. As affected by small quantities of levulose, 389.
- Caskey, Charles (See Heller, V. G.), 59.
- Catherwood, Florence L. (See Rose, William C.), 155.
- Cereal breakfast foods, composition and heat value of, 83.
- — —, nutritive value of, 83, 91, 217.
- Cereals, digestibility *in vitro*, 91.
- , rate of absorption of, in rats, 217.
- , rate of digestion of, in rats, 217.
- Chemistry of vitamin D, 517.
- Chickens, white leghorn, surface area of, 443.
- Children, protein metabolism of, on diets extremely low in carbohydrate, 31.
- Cholesterol, in blood plasma, relation to diet, 77.
- Clausen, Ethel M. Luce, The use of isolated radiations in experiments with the rat. I. The effect of infra-red radiation on the growth of the rachitic rat, 125.

- Clayton, Mary M., The comparative value of different food proteins for reproduction and lactation in the rat. I. Beef muscle, liver and kidney, 491.
- Composition, chemical, of rats raised on different diets, 241, 454.
- Copper, effect of on hemoglobin of sows, 281.
- , retention by white rats on different kinds of milk, 456.
- Corn, vitamin A content of yellow and white-capped yellow dent, 265.
- Cox, Gerald J., and Leona Hudson, The nephropathogenic action of custine. II. The dietary control of cystine nephrosis, 271.
- Creatine, origin of, 433.
- Cyclic variations in the basal metabolic rate of women, 203.
- Cystine deficiencies, application of paired-feeding method in nutrition to problem of, in food proteins, 225.
- , nephropathogenic action of, 271.
- , nephrosis, dietary control of, 271.
- DAHLE, CHESTER D.** (See Honeywell Hannah E., and Dutcher, R. Adams), 251.
- Daniels, Amy L., Dorothy Jordan, and Mary K. Hutton, The development of the suckling young of milk fed rats, 19.
- Dextrose, effect of on gaseous exchange in human subject, 375.
- Diet(s), certain, effect of, on growth and form of albino rats, 7.
- , comparative study of liquid and dry milk as anemia-producing, 451.
- , effect of varying amounts of menhaden oil in, 509.
- , high magnesium, effect of, on growth and metabolism, 471.
- , low calcium, effect of, on growth and metabolism, 471.
- , low fat, influence of, upon fat metabolism during lactation, 67.
- , low in carbohydrate, protein metabolism of children on, 31.
- , rolled oats and bran in a scurvy-producing, 171.
- Dietary control of cystine nephrosis, 271.
- requirements for fertility and lactation. XXII. Further studies of the role of milk fat in fertility and lactation, 485.
- Digestibility of nitrogen, coefficients of, in rats, 240.
- Digestion of starch in cereals, 97, *et seq.*
- peptic, or cereals, 101, *et seq.*
- , tryptic, of cereals, 103, *et seq.*
- Dimit, B. H. (See Quinn, E. J. and King, C. G.), 7.
- Dow, O. D. (See Supplee, G. C., Flanagan, G. E., and Kahlenberg, O. J.), 451.
- Dutcher, R. Adams (See Honeywell, Hannah E., and Dahle, Chester D.), 251.
- EDITORIAL** Review, The emptying mechanism of the stomach, 311.
- , Biological values and the behavior of food and tissue proteins, 419.
- , The chemistry of vitamin D, 517.
- Elvehjem, C. A. (See Hart, E. B., and Steenbock, H.), 277.
- Ergosterol, absorption spectrum of, 521, *et seq.*
- , characteristics of, as affected by irradiation, 527.
- formula for, 519.
- Evans, H. M., and Samuel Lepkovsky, Technique for determination of the anti-neuritic vitamin B, 1.
- Falcon-Lesses, Mark, The cause of the laxative action of bran, 295.
- FAT**, influence of fiber on, in the feces of human subjects, 187.
- , metabolism during lactation, the influence of a low-fat diet upon, 67.
- , of body of white rat as affected by menhaden oil, 509.
- , percentage in milk in relation to fat of diet, 75, *et seq.*
- , yield in milk, relation to diet, 79.
- Fatty acids, highly unsaturated, storage of, 509.
- , in blood plasma, relation to fat of diet, 77.
- Feces, influence of fiber on fat in, of human subjects, 187.
- , production of in rats, as affected by bran, 300, *et seq.*
- Fertility, dietary requirements for, 485.
- , role of milk fat in, 485.
- Fiber, crude, of bran, effect on fecal production, 307.
- , influence of on nitrogen balance and on

- fat in the feces of human subjects, 187.
- Filters, light, care of, 130.
- , —, characteristics of, 122.
- Flanigan, G. E. (See Supplee, G. C., Dow, O. D., and Kahlenberg, O. J.), 451.
- Food, influence of, upon longevity, 415.
- , proteins, biological value of, 419.
- Fox, Edward L. (See Carpenter, Thorne M.), 359, 375, 389.
- G**ASEOUS exchange of the human subject, I.
- As affected by the ingestion of water at 37.0° C., 359.
- , II. As affected by small quantities of dextrose, 375.
- , III. As affected by small quantities of levulose, 389.
- Growth, Do baking powder residues exert injurious effects upon, 155.
- , effect of low calcium, high magnesium diets on, 471.
- , of calf, as affected by magnesium phosphate, 472.
- , of cow, as affected by magnesium phosphate, 473.
- , of mice on vitamin-deficient diets, 54.
- , of rabbits on diets deficient in vitamins A and D, 261.
- , of rat, rachitic, effect of infra-red radiation on, 125.
- , of rats treated with infra-red light, 135, 142, with green light, 136.
- , of suckling young of milk fed rats, 21, *et seq.*
- , of young rats consuming free cystine, 273.
- , records of rats on different kinds of corn, 268.
- , restriction of by deficient diets, 13.
- Growth curves of heifers on different diets, 474.
- — of white rats on different milk diets, 462, *et seq.*
- H**ANN, R. M. (See Schwartz, E. W., and Murphy, F. J.), 171, 325.
- Hart, E. B., C. A. Elvehjem, and H. Steenbock, A study of the anemia of young pigs and its prevention, 277.
- Heat production after ingestion of levulose, 399, *et seq.*
- — after ingestion of water, 368, *et seq.*
- Heat value, of cereal breakfast foods, 83.
- —, physiological, of cereal foods, 89.
- Heller, V. G., and Charles Caskey, An application of some of the more recent methods of estimating vitamin D, 59.
- Hemoglobin, curves of, in white rats on different milk diets, 462, *et seq.*
- , effects of iron upon, 281, 285, 288.
- Hitchcock, F. A., and Frances R. Wardwell, Cyclic variations in the basal metabolic rate of women, 203.
- Honeywell, Hannah E., R. Adams Dutcher, and Chester D. Dahle, Ossifying potency of raw and evaporated milks, 251.
- Hudson, Leona (See Cox, Gerald J.), 271.
- Huffman, C. F., C. S. Robinson, O. B. Winter and R. E. Larson, The effect of low calcium, high magnesium diets on growth and metabolism, 471.
- Hughes, J. S., H. F. Lienhardt, and C. E. Aubel, Nerve degeneration resulting from avitaminosis A, 183.
- Hutton, Mary K. (See Daniels, Amy L., and Jordan, Dorothy), 19.
- I**NSULIN, effect of, upon body weight of the rabbit, 245.
- Iodine number of milk fat, relation to diet, 76.
- Iron, effect of, on hemoglobin of growing sows, 281.
- intake by rats on different kinds of milk, 458.
- retention by white rats on different kinds of milk, 456.
- J**ONES, LOYD A., Isolation of narrow spectral regions by selective absorption, 111.
- Jordon, Dorothy (See Daniels, Amy L., and Hutton, Mary K.), 19.
- K**AHLENBERG, O. J. (See Supplee, G. C., Dow, O. D., and Flanigan, G. E.), 451.
- Ketosis, relationship of, to protein requirement, 38, *et seq.*
- Kidney, comparative value of, for reproduction and lactation, 491.
- , weights of, in rats consuming free cystine, 273.
- King, C. G. (See Quinn, E. J., and Dimit, B. H.), 7.
- Krenz, Carl (See Moritz, Alan R.), 257.
- L**ACTATION, comparative value of different food proteins for, 491.
- , dietary requirements for, 485.
- , influence of low-fat diet upon fat metabolism, during, 67.

- Lactation, role of milk fat in, 485.
- Larson, R. E. (See Huffman, C. F., Robinson, C. S., and Winter, O. B.), 471.
- Laxative action of bran, cause of, 295.
- Lepkovsky, Samuel (See Evans, H. M.), 1.
- Levulose, effect on gaseous exchange in human subject, 389.
- Lienhardt, H. F. (See Hughes, J. S., and Aubel, C. E.), 183.
- Line, Willard R. (See Murlin, John R., Piper, H. A., and Pierce H. B.), 83.
- Liver, comparative value of, for reproduction and lactation, 491.
- of pigs, iron and copper content of, 283, 285.
- Long, M. Louisa, and Fritz Bischoff, The effect of insulin upon the body weight of the rabbit, 245.
- Longevity, influence of food upon, 415.
- MACLEOD, FLORENCE L., The chemistry of vitamin D, 517.
- Magnesium, high, effect of, on growth and metabolism, 471.
- phosphate, effect of feeding, 471.
- Malt diastase, digestion of starch by, in cereals, 106, *et seq.*
- Mattill, H. A., and H. G. Smith, The nutritive value of cereal breakfast foods. III. The rate of digestion and absorption as determined by experiments on rats, 217.
- Maynard, L. A., and C. M. McCay, The influence of a low-fat diet upon fat metabolism during lactation, 67.
- McCay, C. M. (See Maynard, L. A.), 67.
- McLaughlin, Laura, The nutritive value of New Zealand spinach, 197.
- McQuarrie, Irvine, Protein metabolism of children on diets extremely low in carbohydrate, 31.
- Mechanism, emptying, of the stomach, 311.
- Mendel, Lafayette B. (See Bing, Franklin C.), 49.
- Menhaden oil, effect of, in the diet on the composition of the body fat, 509.
- Menstrual period, influence of, on basal metabolism, 206, *et seq.*
- Metabolism, basal, cyclic variations in, of women, 203.
- , fat, influence of low fat diet upon, during lactation, 67.
- , mineral, effect of low calcium, high magnesium diets on, 471.
- , protein, of children on diets low in carbohydrate, 31.
- Methods of estimating vitamin D, an application of some of the more recent, 59.
- of study of digestion *in vitro*, 91.
- Milk, development of suckling young of rats fed on, 19.
- , ossifying potency of raw and evaporated, 251.
- , role of fat of in fertility and lactation, 485.
- , studies on the destruction of vitamin C in the boiling of, 325.
- , study of liquid and dry, as anemia-producing diets, 451.
- , yield, relation of diet to, 70, *et seq.*
- Mineral balance of calf and cows, as affected by magnesium carbonate, 478, *et seq.*
- of heifers as affected by magnesium phosphate, 475, *et seq.*
- Mineral content of New Zealand spinach, 198.
- Mitchell, H. H., The significance of surface area determinations, 437.
- , The surface area of single comb white leghorn chickens, 443.
- , and Jessie R. Beadles, The paired-feeding method in nutrition experiments and its application to the problem of cystine deficiencies in food proteins, 225.
- Moritz, Alan R., and Carl Krenz, The relation of the fat-soluble vitamins A and D to the development of experimental rickets in rabbits, 257.
- Morris, S. G. (See Brown, J. B.), 509.
- Mouse, albino, vitamin B and vitamin G requirements of, 49.
- Murlin, John R., The emptying mechanism of the stomach, 311.
- (See Carman, J. S., Smith, H. G., and Havens, Gladys C.), 91.
- , Willard R. Line, H. A. Piper, and H. B. Pierce, The nutritive value of cereal breakfast foods. I. Composition and heat value, 83.
- Murphy, F. J. (See Schwartze, E. W., and Hann, R. M.), 171, 325.
- NEPHROSIS, the dietary control of in cystine, 271.
- Nerve degeneration resulting from a vitaminosis A, 183.
- Nitrogen balance, influence of fiberon, 187.

- Nitrogen balance of children on different levels of protein 34, *et seq.*  
—, use of in determining biological value of protein, 422.
- Nutrition, Do baking powder residues exert injurious effects upon growth and, 155.  
—, paired-feeding method in, 225.
- Nutritional polyneuritis, experimental, in the rat, 409.
- Nutritive value of cereal breakfast foods,  
I. Composition and heat value, 83.  
— II. Digestibility *in vitro*, with a study of methods, 91.  
— III. The rate of digestion and absorption as determined by experiments on rats, 217.  
— of New Zealand spinach, 197.
- OATS rolled, and bran in a scurvy-producing diet and the negative control test, 171.
- Oils of body of white rat, characteristics of, 513.
- PAIRED-FEEDING method in nutrition experiments and its application to the problem of cystine deficiencies in food proteins, 225.
- pH of feces, method, 60.  
—, relation to rickets, 62.
- Phytin, relation of, to laxative action of bran, 305.
- Pierce, H. B. (See Murlin, John R., Line, Willard R., and Piper, H. A.), 83.
- Pigs, study of the anemia of, and its prevention, 277.
- Piper, H. A. (See Murlin, John R., Line, Willard R., and Pierce, H. B.), 83.
- Polynuritis, experimental nutritional, in the rat, 409.  
—, symptoms of, in the rat, 411.
- Protein(s), biological values and behavior of, in metabolism, 419.  
—, food, comparative value of, in reproduction and lactation, 491.  
— metabolism of children on diets extremely low in carbohydrate, 31.  
—, paired-feeding method in nutrition experiments with, 225.
- QUINN, E. J., C. G. King, and B. H. Dimit, A study of the effect of certain diets upon the growth and form of albino rats, 7.
- RABBIT(s), effect of insulin upon the body weight of, 245.  
—, relation of the fat-soluble vitamins A and D to development of rickets in, 257.
- Radiation(s), infra-red effect of, on the growth of the rachitic rat, 125.  
—, the use of isolated, in experiments with the rat, 125.
- Rat(s), albino, effect of certain diets upon growth and form of, 7.  
—, development of suckling young of milk fed, 19.  
—, experimental nutritional polyneuritis in, 409.  
—, use of isolated radiations in experiments with. I. The effect of infra-red radiation on the growth of the rachitic, 125.
- Reproduction, comparative value of different food proteins for, 491.
- Respiratory quotient(s) after ingestion of dextrose, 377, *et seq.*  
—, after ingestion of levulose, 390.  
—, after ingestion of water, 361.  
—, significance of, after ingestion of levulose, 404.
- Rice, polished, study of the vitamin B complex of, 353.
- Rickets, effect of infra-red radiation on the growth of the rat with, 125.  
—, relation of the fat-soluble vitamins A and D to development of, in rabbits, 257.
- Robinson, S. C. (See Huffman, C. F., Winter, O. B., and Larson, R. E.), 471.
- Rose, William C., and Florence L. Catherwood, Do Baking Powder Residues exert injurious effects upon growth and nutrition? 155.
- Russell, Walter C., The vitamin A content of yellow and white-capped yellow dent corn, 265.
- SALIVA, digestion of starch in cereals by, 96, *et seq.*
- Sandels, M. R., Experimental nutritional polyneuritis in the rat, 409.
- Schwartz, E. W., F. J. Murphy, and R. M. Hann, Rolled oats and bran in a scurvy-producing diet and the negative control test, 171.  
—, —, —, Studies on the destruction of vitamin C in the boiling of milk, 325.



- Scurvy, rolled oats and bran in, 171.
- Season, influence of, on basal metabolic rate, 213.
- Sherman, H. C., and H. L. Campbell, Further experiments on the influence of food upon longevity, 415.
- Smith, H. G. (See Carman, J. S., Havens, Gladys C., and Murlin, J. R.), 91.
- (See Mattill, H. A.), 217.
- Specific dynamic action of dextrose, 383.
- — — of levulose, 403.
- Spectral regions, narrow, isolation of, by selective absorption, 111.
- Spectrograms of liquid milk ash and dry milk ash, 461.
- Spectrophotometric absorption curves for light filters, 118, 120.
- Spinach, New Zealand, nutritive value of, 197
- Spinal cord, microphotograph showing degeneration in, 186.
- Steenbock, H. (See Hart, E. B., and Elvehjem, C. A.), 277.
- Stomach, emptying mechanism of, 311.
- Supplee, G. C., O. D. Dow, G. E. Flanigan, and O. J. Kahlenberg, A comparative study of liquid and dry milk as anemia-producing diets, 451.
- Sure, Barnett, Dietary requirements for fertility and lactation, XXII. Further studies of the role of milk fat in fertility and lactation, 485.
- Surface area determinations, significance of, 437.
- — — formulae of the chicken, 447.
- — — of rats placed in different positions, 441.
- — — of right and left halves of chickens, 438, *et seq.*
- — — of single comb white leghorn chickens, 443.
- THOMAS, KARL, Biological values and the behavior of food and tissue proteins, 419.
- Tissue proteins, behavior of, in metabolism, 419.
- UTILIZATION of nitrogen and fat, effect of fiber on, 191.
- VITAMIN A content of yellow and white-capped yellow dent corn, 265.
- — —, nerve degeneration resulting from lack of, 183.
- — —, relation of to the development of experimental rickets in rabbits, 257.
- — —, tests for in spinach, 200.
- Vitamin B requirements of the albino mouse, 49.
- — —, study of the complex of, in red kidney beans and polished rice, 353.
- — —, tests for in spinach, 201.
- Vitamin C, methods of assay, 327.
- — —, studies on the destruction of, in the boiling of milk, 325.
- Vitamin D, an application of some of the more recent methods of estimating, 59.
- — —, chemistry of, 517.
- — —, relation of, to the development of experimental rickets in rabbits, 257.
- Vitamin E, in relation to protein feeding, 498.
- Vitamin G, picture of deficiency in mice, 57.
- — — requirements of the albino mouse, 49.
- Vitamin studies XVII. Ossifying potency of raw and evaporated milks, 251.
- WARDWELL, FRANCES R. (See Hitchcock, F. A.), 203.
- Water ingestion, effect on gaseous exchange in human subject, 359.
- Whitacre, Jessie, Alice Willard and Katherine Blunt, Influence of fiber on nitrogen balance and on fat in the feces of human subjects, 187.
- Willard, Alice (See Whitacre, Jessie, and Blunt, Katherine), 187.
- Winter, O. B. (See Huffman, C. F., Robinson, C. S., and Larson, R. E.), 471.
- Women, cyclic variations in the basal metabolic rate of, 203.





IMPERIAL AGRICULTURAL RESEARCH  
INSTITUTE LIBRARY  
NEW DELHI.

Date of issue.	Date of issue.	Date of issue.
11.7.39		
22.9.43		
25.4.49		